University of Alberta

LIBRARY RELEASE FORM

Name of Author: Sarah Jane Robbins

Title of Thesis: North American ginseng and the immune response during acute exercise

Degree: Master of Science

Year this Degree Granted: 2001

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

University of Alberta

North American ginseng and the immune response during acute exercise

By

Sarah Jane Robbins ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of Master of Science
in
Nutrition and Metabolism
Department of Agricultural, Food, and Nutritional Sciences

Edmonton, Alberta
Fall, 2001

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **North American ginseng and the immune response during acute exercise**, submitted by Sarah Jane Robbins in partial fulfillment of the requirements for the degree of Master of Science in Nutrition and Metabolism.

This work is dedicated to:

Mum and Dad,
who taught me to pursue excellence

Akasha,
who encourages me to pursue my dreams

Michael and Tim,
who teach me to laugh.

Abstract

Ten moderately active adult males (mean VO_{2max} 44.5 ml/kg/min) completed this double-blind, placebo-controlled, within-subject cross-over study to determine the effects of 35 days of daily supplementation with 1125 mg North American ginseng extract on immune responses to acute exercise (15 minutes at 80% ventilatory threshold and 15 minutes at 100% ventilatory threshold). Venous blood samples were collected 15 minutes before exercise, immediately after exercise, and after 30 minutes of recovery. Ginseng administration did not alter the number of circulating leukocytes, neutrophils, or lymphocytes at rest; there was no effect of ginseng treatment on neutrophil oxidative burst activity. Following exercise, ginseng consumption results in an increased number of circulating monocytes. Ginseng administration prevented exercise-induced declines in the proportion of CD3+ and CD4+ cells and increased the proportion of CD8+/CD45RO+ and CD16+ lymphocytes following moderate physical stress. The functional capacity of lymphocytes following exercise was increased by ginseng administration, as determined by increased *in vitro* IL-2 production and lymphocyte proliferation. The present study provides evidence that daily consumption of ginseng may alter some aspects of the response to physical stress.

Acknowledgements

Thank-you to Linda McCargar, for your guidance and support in all of my endeavours. Thanks also to Catherine Field and Vicki Harber for your assistance throughout the study.

Thank-you to Sue Goruk and Vera Pratt, without whose expert technical assistance and friendship this thesis would not have been possible. Thank-you to Geoff Ball, for providing me with support and advice on every aspect of my degree. Thank-you to Jennifer Humphreys for so much hard work on this project and for all the fun you brought to the study.

Thank-you to the participants, nurses, and many graduate students and staff at the University of Alberta who contributed their time and energy to the success of this study.

Finally, thank-you to the Chai-Na-Ta Corporation and the the Natural Sciences and Engineering Research Council of Canada for their financial support of this study.

Table of Contents

Chapter One
Introduction

Chapter Two
Literature Review

Chapter Three
Experimental Design and Methodology

Chapter Four
Results

Chapter Five
Discussion

References

List of Appendices

List of Tables

Chapter Three

Chapter Four

List of Figures

Chapter 4

List of Symbols and Abbreviations

ADCC	antibody dependent cytotoxic cell
ANP	atrial natriuretic peptide
BSA	bovine serum albumin
CCM	complete culture media
CD	cluster of differentiation
Con A	concanavalin A
CTL	cytotoxic T-lymphocyte
DHR	dihydrorhodamine 123
ELISA	enzyme linked immunosorbent assay
FCS	fetal calf serum
H2O2	hydrogen peroxide
HOCl	hypochlorous acid
IFN	interferon
IgG	immunoglobulin
IL	interleukin
LCA	leukocyte common antigen
LPS	lipopolysaccharide
mAb	monoclonal antibodies
MPO	myeloperoxidase
NAGE	North American ginseng extract
NK	natural killer cell
NO	nitric oxide
NOS	nitric oxide synthase
O2-	superoxide
PBS	phosphate buffered saline
PHA	phytohemaglutinin
PMA	phorbol myristate acetate
PMN	polymorphonuclear leukocytes
PWM	pokeweed mitogen
RER	respiratory exchange ratio
ROS	reactive oxygen species
RV	residual lung volume
SOD	superoxide dismutase
SI	stimulation index
URTI	upper respiratory tract infection
TCR	T-cell receptor
TNF	tumor necrosis factor
VO_{2max}	maximal ventilatory oxygen consumption
VT	ventilatory threshold

Chapter One

Introduction

A. Rationale

Ginseng is an herbal root known as the panacea of Chinese medicines. There are three species of ginseng recognized to have medicinal properties: *Panax ginseng* C.A. Meyer (Chinese or Korean ginseng, grown in mountainous forests of Asia), *Panax japonicus* C.A. Meyer (grown in Japan, India, and Southern China), and *Panax quinquefolius* (North American ginseng, grown in eastern and central Canada and the United States; Bahrke and Morgan, 1994). Siberian or Russian ginseng is claimed to have similar properties as the *Panax* species, however it belongs to the *Eleutherococcus* family (Bahrke and Morgan, 1994). *Panax* species differ in their content of active constituents known as ginsenosides (also termed triterpenoid glycosides, saponins, and panaxosides; Bahrke and Morgan, 1994).

Ginseng has been included in Chinese pharmacology for over 2,000 years; it has traditionally been used to cure digestive problems, nervous disorders, fevers, reduce inflammation and even prolong life (Goldstein, 1975). The herb is believed to restore and maintain the balance of Yin and Yang in the body and is often used in combination with other herbs to cure a large variety of ailments. North American ginseng has a long history of use among many Aboriginal groups (Goldstein, 1975). It was first discovered by European settlers in the early 1700's; by the 1720's the ginseng trade between China

and North America was established (Goldstein, 1975). At this time, ginseng was the second most traded commodity in Canada (Goldstein, 1975).

Recent interest in ginseng and other herbal products has stemmed from an increasing concern about food additives and pharmaceuticals and the belief that natural products are healthy and safe (Dubick, 1986). Recent estimates indicate that at least 6 million Americans use ginseng to treat various ailments (O'Hara et al., 1998). In spite of the current popularity of herbal products, few have undergone standardization, quality control, or commercial regulation (Wong et al., 1998).

Ginseng is considered to be an adaptogen that increases resistance to stress and promotes vitality (O'Hara et al., 1998; Bahrke and Morgan, 1994). Recent scientific studies suggest that *Panax* species have both stimulatory and inhibitory effects on the central nervous system, cardiovascular effects, and antineoplastic and immunomodulatory effects (Attele et al., 1999). North American ginseng has been shown *in vitro* to increase lymphocyte proliferation (Borchers et al., 1998). A purified form of the ginsenoside Rg1 has been shown to enhance nitric oxide production from activated macrophages *in vitro* (Fan et al., 1995). *In vitro* studies have indicated that ginseng augments natural killer (NK) cell and antibody dependent cytotoxic (ADCC) activities (Singh et al., 1994). In murine models, supplementation with ginseng has been shown to amplify host resistance to viral infection (Singh et al., 1983). Administration of *Panax ginseng* to humans has been shown to potentiate vaccination against the common cold and influenza viruses, increase antibody titers, increase phagocytic activity of

polymorphonuclear leukocytes (PMN), increase the ratio of CD4/CD8 T-cells, and increase NK cell activity (Scaglione et al., 1990; Scaglione et al., 1996). Conversely, Srisurapanon et al. (1997) found no effect of *Panax ginseng* administration on peripheral blood leukocytes of lymphocyte subsets. Few studies have been conducted on the immunomodulatory effects of North American ginseng (Borchers et al., 1998).

Several experimental models exist to determine the effects of physical stress on immunological responses of humans; these include hypoxia, head up tilt (simulating hemorrhagic shock), hyperthermia, and various types of physical exercise (Hoffman-Goetz and Pedersen, 1994). It is thought that the immune response to exercise model has potential clinical applications in the understanding and treatment of cancer, HIV/AIDS infection, autoimmune disorders, aging, and even immunosuppression during spaceflight (Mackinnon, 1999). For example, exercise has been linked to a reduced risk of all-cause and colonic cancers; this risk reduction may in part be due enhanced natural killer cell function, enhanced macrophage chemotaxis, or modified levels of cytokines that occur with regular exercise (Shepard and Shek, 1998). It is hypothesized that regular, moderate exercise may stimulate immune function and therefore result in the early detection and elimination of cancerous cells (Shepard and Shek, 1998). Thus, exercise protocols can be used to elicit a physical stress response.

B. Purpose

The purpose of the current research is to investigate the effects of daily consumption of North American ginseng, *Panax quinquefolius*, on the immune response

to acute physiological stress (a moderate exercise protocol). A double-blind, randomized, placebo-controlled cross-over design was used to reduce bias and control for variability in the immune response. Ten healthy adult males participated in two treatment periods, separated by a three-month washout phase. Indices of aerobic fitness, body composition, dietary intake, peripheral leukocyte counts, lymphocyte subsets, lymphocyte proliferation, neutrophil function, and interleukin-2 production were assessed at the end of each ginseng or placebo treatment period.

A paucity of research exists on the effects of North American ginseng administration on immune function in humans. This study provides insight into the effects of this popular herb on the immune response to physical stress and contributes to the growing body of literature on the efficacy and safety of herbal products. This information will be useful in the evaluation of the therapeutic potential and safety of North American ginseng.

C. Hypotheses

1. Ginseng administration will result in a reduced exercise leukocytosis compared to placebo treatment.
2. Ginseng administration will reduce exercise-induced changes in lymphocyte subsets compared to placebo treatment.
3. Ginseng administration will increase the ability of mitogen-stimulated lymphocytes to proliferate *in vitro* compared to placebo treatment.

4. Ginseng administration will increase neutrophil oxidative burst compared to placebo treatment.

5. Ginseng administration will increase the interleukin-2 production of lymphocytes compared to placebo treatment.

D. Objectives

1. To implement a significant, yet safe, physiological stress using a standardized exercise protocol.

2. To determine for descriptive purposes the aerobic fitness, physical activity scores, body composition, and dietary intake using VO_{2max}, the modified Baecke Questionnaire, underwater weighing, and three-day food records, respectively.

3. To determine if regular consumption of North American ginseng reduces an individual's response to a physical stress as determined by leukocyte number, lymphocyte proliferation, neutrophil oxidative burst, and lymphocyte interleukin-2 production.

Chapter Two

Literature Review

A. Introduction

Exercise has been shown to be a powerful modulator of the activity of the immune system. This literature review will focus on the effects of acute exercise on the immune system and provide the background rationale for the use of exercise as an experimental stressor of the immune system. The properties of ginseng will be reviewed, followed by a review of the current literature exploring the effects of ginseng on the immune system.

B. Exercise Immunology

Reports of exercise-induced leukocytosis have been written for over 100 years (Pedersen et al., 1997; Gabriel and Kindermann, 1997). Recent interest in the effects of exercise on immune function has been influenced by reports that athletes experience increased frequencies of upper respiratory tract infections, the hypothesis that muscle metabolism is linked to immune function, the possible roles of neuroendocrine factors on the immune system, and the finding that physical stressors, including surgery or trauma, modulate the immune system (Pedersen et al., 1997). It is therefore likely that exercise can serve as a model of the immune response to physical stress. It is hoped that a greater understanding of the immune response to exercise will have potential clinical applications in the prevention and treatment of disease. For example, it is hypothesized that exercise may lead to a reduced risk of colorectal cancer through the stimulation of

the immune system by regular moderate exercise (Mackinnon, 1999; Shepard and Shek, 1998). Limited data suggest that regular moderate exercise may enhance survival, quality of life, and immune function in individuals affected by HIV (Mackinnon, 1999). Research into the effects of various intensities and durations of exercise on immune function may therefore be used to develop exercise prescriptions for people affected by these diseases that improve quality of life without causing undue harm (Mackinnon, 1999). In addition, little data exists to provide training recommendations to athletes who are experiencing infections (Nieman, 1997). Currently, it is suggested that athletes resume training only if they are free of systemic symptoms (Nieman, 1997). Research into exercise and immunity may prove valuable in reducing the morbidity and mortality associated with common illnesses in our society.

Recent research has greatly increased our understanding of the relationship between exercise, immune function and human health (Nieman, 1997). Many reports have demonstrated that changes in circulating immune cell numbers and function are dependent on both the intensity and duration of exercise (Smith, 1997; Nieman, 1997). Longer and more intense periods of exercise result in a more profound neutrophilia and lymphocytopenia than moderate exercise bouts (Gabriel and Kindermann, 1997; Nieman, 1997). A variety of mechanisms may be involved in modulating the immune response to exercise, including changes in stress hormones, cytokines, body temperature, blood flow, and hydration status (Nieman, 1997). Additionally, the physical fitness levels of research participants have been shown to influence the effects of exercise on the immune system (Neiman, 1997). Highly trained athletes have been reported to have

enhanced natural killer cell activity and suppressed neutrophil function compared to sedentary controls (Nieman, 1997). Generally, fewer differences in mitogen-induced lymphocyte proliferation (a measure of T-cell function) and immunoglobulin levels have been demonstrated between athletes and nonathletes in response to exercise (Nieman, 1997). Nieman (1997) suggests that overall the innate immune system responds to chronic exercise to a greater degree than the adaptive immune system. Moderate training (defined as 3-5 sessions/week, 15-60 minutes/session, 40-60% VO_{2max}) has a lesser effect on the immune system, whereas more intense training appears to be immunosuppressive (Shepard and Shek, 1999). It has been proposed that the relationship between exercise training and the risk of upper respiratory tract infection (URTI) be modeled in the form of a "J" curve, indicating that moderate exercise training decreases the risk of URTI below that of a sedentary individual while excessive amounts of high-intensity training increase the risk of URTI far above average (Shepard and Shek, 1999; Nieman, 1997; Hoffman-Goetz and Pedersen, 1994).

1. Responses of Neutrophils and Total Leukocytes to Acute Moderate Exercise

Within the first 30 minutes of a bout of moderate exercise, leukocyte subpopulations begin to rise (Gabriel and Kindermann, 1997). Tvede et al. (1993) demonstrated that total leukocyte numbers rise during and for two hours after a moderate exercise protocol in healthy young men (mean VO_{2max} of 55.6 ml/kg/min). During exercise, this leukocytosis is due to both increases in lymphocytes and neutrophils (Tvede et al., 1993; Smith, 1997). It is at about 30 minutes of exercise that lymphocytes and monocytes have been reported to reach maximal values (Gabriel and Kindermann, 1997).

However, the leukocytosis during the recovery period is due primarily to a further increase in neutrophils with a decline in the number of circulating lymphocytes (Tvede et al., 1993). Upon cessation of exercise, lymphocyte subpopulations and mature monocytes (CD14+, CD16+) in peripheral blood begin to decline in number (Gabriel and Kindermann, 1997); these values dip below baseline within the first two hours of exercise cessation (Gabriel and Kindermann, 1997). Leukocytes are thought to be mobilized from the lung, bone marrow, and vascular systems during and after exercise; the spleen is thought to be of minor importance in the demargination of leukocytes with exercise (Baum et al., 1996).

Neutrophils (also known as polymorphonuclear leukocytes) normally represent 50-60% of the circulating leukocyte pool and are essential to host defence (Smith, 1997). When an inflammatory cascade is initiated by an infectious agent or non-self substance (bacteria, fungi, protozoa, viruses, virally-infected cells, or tumor cells, for example), neutrophils are recruited to the site of infection via the generation of chemotactic gradients produced by other components of the immune system or by infectious agents (Smith, 1997). Once recruited to the site of infection, neutrophils adhere to the vascular endothelium, penetrate the endothelium and move towards the infectious organism or non-self cell in a process known as diapedesis (Smith, 1997). Finally, neutrophils congregate at this site and adhere to the extracellular matrix components, including laminin and fibronectin (Smith, 1997). Neutrophils function as effector cells, as well as synthesize and secrete humoral mediators such as cytokines (Smith, 1997). Microorganisms or non-self cells are recognized via opsonization or through nonspecific

receptors that recognize certain lectins and are subsequently phagocytized by neutrophils (Smith, 1997). Upon phagocytosis, the oxidative burst and degranulation processes are activated. The oxidative burst consists of a 50– to 100- fold increase in oxygen consumption and resultant production of cytotoxic reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl; Smith, 1997). The NADPH oxidase system, which is inactive in dormant neutrophils, catalyzes the formation of superoxide from molecular oxygen (Smith, 1997). The enzyme superoxide dismutase (SOD) is involved in the formation of H_2O_2, a potent ROS. H_2O_2 may be further combined with Cl^- via the myeloperoxidase (MPO) enzyme to form oxyhalides such as HOCl (Smith, 1997). The degranulation process consists of the release of contents of primary (azurophilic) and secondary (specific) granules into the phagocytic vesicle to form a phagolysosome (Smith, 1997). Hydrolytic enzymes in neutrophils serve to augment microbial damage caused by ROS and to digest engulfed infectious organisms or non-self cells (Smith, 1997). Neutrophils are regulated through a variety of counterregulatory and redundant pathways which are controlled by humoral mediators and direct cell-to-cell contact (Smith, 1997). Soluble mediators may be of endocrine, neurologic, cardiovascular, or immune origin and include cytokines, hormones, and bioactive lipids (Smith, 1997). Neutrophils may cause host tissue damage through several mechanisms, including premature activation during chemotaxis, extracellular release of microbicidal products, and failure to terminate the acute inflammatory response (Smith, 1997). Thus, neutrophils make a significant contribution to the pathology of inflammatory conditions, making it unclear whether exercise-induced

alterations in neutrophil number and function have positive or negative consequences for the individual (Smith, 1997).

Neutrophil concentrations increase within the first 30 minutes of exercise, and continue to increase for the first two hours after exercise cessation (Gabriel and Kindermann, 1997). Neutrophilia predominates over lymphocytopenia once cortisol release is activated at about 60% of VO_{2max} (Smith, 1997). The functional activity of neutrophils includes both microbicidal production of ROS and phagocytosis of non-self particles; these parameters may respond differentially to various intensities and durations of exercise and may not correspond to changes in circulating neutrophil numbers (Smith, 1997). The oxidative burst activity, phagocytosis and killing capacity appear to be increased following a bout of moderate exercise (1 hour of cycling at 60% VO_{2max}; Smith, 1997). In contrast, strenuous exercise appears to result in a decrease in neutrophil oxidative burst activity (Gabriel and Kindermann, 1997; Smith, 1997). It is hypothesized that during moderate exercise, growth hormone, cytokines, and atrial natriuretic peptide (ANP) mediate the priming and activation of neutrophils (Smith, 1997). During and for some hours following strenuous exercise, greater levels of adrenalin and cortisol are secreted, resulting in a suppression of neutrophil microbicidal activity (Smith, 1997). Increases in neutrophil numbers during and after exercise appear to be related to increases in serum cortisol; both of the parameters are only mildly elevated by moderate exercise (Gabriel and Kindermann, 1997). At higher intensities or durations of exercise, the increase in cortisol and neutrophil numbers is accompanied by decreases in neutrophil activity (as measured by oxidative burst and phagocytosis;

Gabriel and Kindermann, 1997). The effects of moderate exercise on neutrophil function are less clear (Gabriel and Kindermann, 1997). It appears that moderate exercise shifts a proportion of neutrophils from a quiescent to a responsive state and amplifies the activity of responsive neutrophils, whereas intense exercise may lead to a suppression of the activity of a large portion of circulating neutrophils (Smith, 1997).

2. Responses of Monocytes to Acute Moderate Exercise

The mononuclear phagocytic system involves both circulating monocytes and tissue macrophages (Kuby, 1999). Monocytes are produced in the bone marrow and enter the blood stream, where they circulate and enlarge; eventually, monocytes migrate into various tissues and differentiate into tissue macrophages (Kuby, 1999). Once in the tissues, macrophages may be free and move throughout the tissue or they may become fixed and perform specific functions based on their location (Kuby, 1999). Macrophages are attracted to a variety of particulate antigens, including whole microorganisms, insoluble particles, injured and dead host cells, and cellular debris (Kuby, 1999). Macrophages phagocytose these particles into phagolysosomes containing hydrogen peroxide, oxygen-free radicals, and a variety of hydrolytic enzymes in order to digest the particle (Kuby, 1999). The phagocytic activity of macrophages can be enhanced by cytokines secreted from other immune cells, particularly interferon-γ secreted by Th1 cells (Kuby, 1999). In addition to their phagocytic activity, macrophages function to present antigens to T helper cells (Kuby, 1999).

Monocytes are easily studied in peripheral blood during exercise protocols, whereas their macrophage counterparts are difficult to sample from human tissue; this review will therefore explore only the effects of exercise on peripheral monocytes. Peripheral blood consists of both circulating regular monocytes (CD14+, CD16-) and mature monocytes (CD14+, CD16+; Gabriel and Kindermann, 1997). Moderate exercise slightly increases blood levels of regular monocytes, although to a lesser degree than short, intense exercise or than prolonged exercise (Gabriel and Kindermann, 1997); minimal changes in monocyte numbers occur following moderate exercise (Gabriel and Kindermann, 1997). For example, in untrained males (mean VO_{2max} of 55.6 ml/kg/min) exercise at 25%, 50%, and 75% of VO_{2max} had little impact on the number of circulating monocytes; only exercise at 75% of VO_{2max} resulted in an increase in monocytes after two hours of recovery (Tvede et al., 1993). In a study of sedentary young men (mean VO_{2max} of 48.0 ml/kg/min), Shinkai et al. (1996) demonstrated an increase in monocytes during exercise and for seven hours of recovery after a moderate exercise bout of 60 minutes of cycling at 60% VO_{2max}.

3. Responses of Natural Killer Cell and Lymphocyte Subpopulations to Acute Moderate Exercise

During hematopoeisis the bone marrow produces a variety of immune cells, including lymphocytes. There are several broad classes of lymphocytes: B-cells, T-cells, and natural killer (NK) cells. Lymphocytes form 20-40% of the body's leukocytes and cannot be distinguished morphologically; cell-membrane components and function form the basis of the classification of lymphocytes (Kuby, 1999). The expression of

membrane molecules recognized by a particular monoclonal antibody form the basis of the cluster of differentiation (CD) nomenclature (Kuby, 1999). B-cells (CD20+) mature in the bone marrow and are subsequently released into the circulation expressing unique membrane-bound antibody molecules (Kuby, 1999). These naïve B-lymphocytes bind antigen specific to their antibody molecules and divide to become memory B-cells and effector B-cells (Kuby, 1999). Memory B-cells have a long life-span and express membrane-bound antibody with the same specificity as the original naïve B-cell; these cells are thought to form the basis of immunological memory (Kuby, 1999). Effector (also known as plasma) B-cells function to secrete antibodies and have a short life-span (Kuby, 1999).

Once produced in the bone marrow, T-cells migrate to the thymus gland to mature and express a unique antigen-binding T-cell receptor (Kuby, 1999). T-cells generally express CD3. In addition, there are two well defined subsets of T-cells: T-helper cells, which express CD4, and T-cytotoxic cells, which express CD8 (Kuby, 1999). Once a T-helper cell interacts with an antigen-major histocompatibility (MHC) II complex, it functions to secrete glycoproteins termed cytokines which further act to activate other cells of the immune system (Kuby, 1999). Once a T-cytotoxic cell has interacted with an antigen-MHC I complex, it becomes a cytotoxic T lymphocyte (CTL) and functions to eliminate cells displaying antigen (Kuby, 1999). These cells include virus-infected cells, tumor cells, and other altered self-cells (Kuby, 1999).

Natural killer cells are large granular lymphocytes that do not express antigen-binding receptors (Kuby, 1999). NK cells mediate cytolytic reactions against neoplastic and virally-infected cells, as well as inhibit the colonization and growth of certain viruses, bacteria, fungi, and parasites (Nieman, 1997b). NK cells normally represent 10-15% of total blood lymphocytes (Nieman, 1997b). NK cells are particularly responsive to exercise (Baum et al., 1996). NK cells (CD3-, CD16+/CD56+) have been shown to increase up to 300% after 30 minutes of moderate exercise and subsequently decline in the post-exercise period (Gabriel and Kindermann, 1997). After 60 minutes of moderate exercise (60% VO_{2max}) in sedentary young males (mean VO_{2max} of 48 ml/kg/min), Shinkai et al. (1996) showed that NK cells increased by up to 800% of pre-exercise levels, with a subsequent decline below baseline levels in the recovery period. Tvede et al. (1993) demonstrated that NK cells (CD16+) increased by up to 100% after exercise of 25%, 50%, and 75% of VO_{2max} in untrained males (mean VO_{2max} of 55.6 ml/kg/min); a simultaneous increase in NK cell activity, determined by target cell lysis, was found in all exercise types. NK cell numbers were depressed after two hours of recovery in all of the exercise groups with a corresponding decrease in NK cell activity (Tvede et al., 1993). Brenner et al. (1999) studied the impact of three different types of exercise (5 minutes of cycling at 90% VO_{2max}, circuit-training routine at 50% VO_{2max}, or 2 hours of cycling at 60% VO_{2max}) on the NK cell count and activity of moderately fit young males. Subjects were followed up to 72 hours after the exercise protocol. All three types of exercise resulted in significant increase in NK cell numbers; NK cell numbers returned to baseline values within three hours of recovery and never dropped below baseline values (Brenner et al., 1999). NK cell cytotoxicity, expressed on a per-cell basis, was not altered

by any of the exercise protocols (Brenner et al., 1999). The total cytolytic activity was increased after both cycling bouts, suggesting that the changes in cytolytic activity are related primarily to changes in NK cell concentration (Brenner et al., 1999). The authors note that exercise performed in the study was vigorous, but not exhausting; previous reports have indicated that exhausting exercise may reduce NK cell cytotoxicity (Brenner et al., 1999; Nieman, 1997b).

Increases in the number of circulating lymphocytes following exercise have been well documented (Hoffman-Goetz and Pedersen, 1994). The fluctuations in the number of CD8+ T-cells are greater than the fluctuations in the number of CD4+ T-cells (Gabriel and Kindermann, 1997). Moyna et al. (1996) studied the effects of cycling for three consecutive exercise stages for six minutes each at 55%, 70%, and 85% of VO_{2max} on lymphocyte numbers and cytokine production in both males and females. They found that relative to baseline and control conditions, exercise resulted in a significant decrease in the percentage of T-cells. The proportion of CD4+ lymphocytes was decreased by exercise; no changes in the proportion of CD8+ lymphocytes were found. The CD4+/CD8+ ratio was decreased throughout exercise; this was due to an increase in the absolute number of CD8+ cells. Moderate exercise (60 minutes at 60% VO_{2max}) in sedentary males (mean VO_{2max} of 48 ml/kg/min) resulted in increased total T-lymphocytes (CD3+), T-helper cells (CD4+), and T-cytotoxic cells (CD8+); CD8+ cells demonstrated the greatest increase in concentration (Shinkai et al., 1996). The recovery period following this exercise protocol was marked by a decrease below pre-exercise values in all three cell types (Shinkai et al., 1996). This lymphopenia took up to 6 hours

to return to baseline values. During the recovery period, the impact of exercise was greatest on the CD4+ population of T-cells and resulted in a decreased CD4/CD8 ratio.

Lymphocyte proliferation *in vitro* has also been used to describe the effects of exercise on T-cells. Generally, cells isolated following cycling exercise have been found to have a decreased proliferative response to phytohemaglutinin (PHA) thought to be due to an alteration in the fraction of responsive CD4+ T-cells (Hoffman-Goetz and Pedersen, 1994). Tvede et al. (1993) demonstrated that cycling for 60 minutes at 25%, 50%, and 75% of VO_{2max} decreased the ability of blood mononuclear cells from untrained men to proliferate *in vitro* in response to PHA; similar results were found on cells collected two hours after recovery from these exercise protocols. These changes were correlated to the decreases seen in the proportion of CD4+ cells (Tvede et al., 1993).

The responses of B-cells to exercise have been less well-studied than the responses of T-cells (Hoffman-Goetz and Pedersen, 1994). Untrained men (mean VO_{2max} of 55.6 ml/kg/min) demonstrated no changes in the proportion of B-lymphocytes following 60 minutes of cycling at 25%, 50%, and 75% of VO2max (Tvede et al., 1993). Similarly, untrained men (mean VO_{2max} of 53.5 ml/kg/min) demonstrated no changes in the number of B-lymphocytes following two hours of cycling at 60% VO_{2max} (Oshida et al., 1988). In another study of sedentary young men (mean VO_{2max} of 48 ml/kg/min), Shinkai et al. (1996) demonstrated small increases in the number of B-cells during a moderated exercise protocol; B-cell numbers returned to pre-exercise levels within 30 minutes of recovery from exercise.

4. Responses of Cytokines to Acute Moderate Exercise

Cytokines are immunoregulatory proteins produced by a variety of immune cells; they have multiple pleiotropic actions on target cells and act to increase or reduce the responses of the immune system (Smith, 1997). The secretion of cytokines by immune cells appears to be dependent on both exercise intensity and duration. Cytokines such as interleukin-1 (IL-1), tumor necrosis factor (TNF), and IL-6 are elevated by more intense or prolonged exercise, but changes in cytokine release during moderate exercise are unclear (Gabriel and Kindermann, 1997). IL are polypeptides that stimulate the growth and differentiation of immune cells (Shepard et al., 1994). The T-helper (CD4+) lymphocyte population is comprised of at least two subsets with differing cytokine production, called Th1 and Th2 (Moyna et al., 1996). Th1 lymphocytes are responsible for the production of IL-2 and interferon-γ (IFN-γ) and contribute to cell-mediated immune functions whereas the Th2 lymphocytes are responsible for the production of IL-4, IL-5, IL-6, and IL-10 and humoral immune functions (Moyna et al., 1996).

As illustrated above, a wide variety of IL are produced by the body; this literature review will focus on the production and action of IL-2. IL-2 is produced mainly by activated T-helper cells and has both paracrine and autocrine functions (Shepard et al., 1994). IL-2 functions to stimulate T-cell maturation, proliferation of T- and B-cells, increase IL-2 receptor expression, release other cytokines, and to stimulate the proliferation and action of NK cells, lymphokine-activated killer (LAK) cells, monocytes, and macrophages (Shepard et al., 1994). The IL-2 receptor (IL-2R) is thought to be comprised of alpha (p 55), beta (p 70-75), and gamma chains (p64), each with differential

expression and binding affinity for IL-2 (Shepard et al., 1994). The alpha and beta receptors act together to form a high-affinity heterodimer, thus allowing IL-2 to transmit signals at concentrations as low as 10^{-15} to 10^{-10} mol/L (Shepard et al., 1994). IL-2/IL-2R binding results in internalization of the complex and cellular proliferation (Shepard et al., 1994). Many T-helper cells (CD4+) express the p55 receptor (CD25) under resting conditions, whereas few T-cytotoxic cells (CD8+) express p55 at rest (Shepard et al., 1994). Conversely a far greater proportion of CD8+ cells and NK cells express the p75 receptor at rest than do CD4+ cells or B-cells and therefore require greater concentrations of IL-2 to proliferate (Shepard et al. ,1994). The stimulation of peripheral blood mononuclear cells with the PHA mitogen results in a 30-100 fold upregulation of the p55 (CD25) receptor, but has little effect on the p75 receptor (Shepard et al., 1994). Plasma concentrations and *in vitro* production of IL-2 appear to have variable responses to physical activity, depending on the intensity and duration of exercise (Shepard et al., 1994). Tvede et al (1993) found that *in vitro* IL-2 production by lymphocytes from untrained men was dependent upon the intensity of exercise. Unstimulated lymphocyte IL-2 production was decreased during exercise at 25% of VO_{2max}, unchanged at 50% of VO_{2max}, and decreased at 75% VO_{2max}. Stimulation of lymphocytes with indomethacin resulted in decreased IL-2 production following exercise at 25% and 75% of VO_{2max}, and increased IL-2 production following exercise at 50% VO_{2max}. The authors postulate that the changes in IL-2 production are related to altered numbers of the IL-2-producing T-helper cells (Tvede et al., 1993).

Overall, the post-exercise period consists of an increase in neutrophils and regular monocytes and a decrease in NK cells, T-cells, B-cells, premacrophages, eosinophils (Gabriel and Kindermann, 1997). The increases in neutrophils and monocytes appear to be due to mobilization from the marginal pool and bone marrow (Gabriel and Kindermann, 1997). While the effects of an acute bout of exercise alter the number of circulating immune cells, the effects on cell function are small relative to changes due to infection (Gabriel and Kindermann, 1997). The clinical relevance of changes in immune parameters due to moderate exercise thus remain unclear (Gabriel and Kindermann, 1997). It is also unclear as to what intensity or duration of exercise could be used to improve immunity and prevent morbidity.

C. Ginseng

1. Ginseng History and Traditional Uses

Ginseng has been regarded as the panacea of all Chinese medicines for over 2,000 years (Hou, 1977; Goldstein, 1975). It has been claimed to cure prostration, heart failure, asthma, shortness of breath, spontaneous sweating, cold limbs, palpitation, long-term debility, neurosis (Chang and But, 1986), consumption diseases, fatigue, thirst, and indigestion (Hou, 1977). The name "ginseng" means "man root" in Chinese and refers to the resemblance of the root to the shape of the human body (Goldstein, 1975). North American Aboriginal groups also used ginseng for similar purposes as the Chinese, including cardiovascular problems, pulmonary difficulties, problems associated with old age, and as an aphrodisiac (Goldstein, 1975). The myths of origin and attitude towards ginseng are remarkably similar between Aboriginal groups and the Chinese (Goldstein,

1975). European settlers discovered ginseng in North America in the early 1700's and quickly established trade with the Chinese (Goldstein, 1975). Today, ginseng is among the most popular herbs in North America, with an estimated six million Americans regularly consuming the herb (O'Hara et al., 1998).

2. Types of Ginseng

There are eight species of *Panax ginseng* that grow within the northern hemisphere: *ginseng C.A. Meyer, quinquefolium, notoginseng, pseudoginseng, zingigerensis, trifolus, stipuleanatus, and japonicus* (Vigano and Ceppi, 1994). *Panax* species all contain the dammarane and oleanolic acid saponins, although the species differ in chemical composition and concentration (Vigano and Ceppi, 1994). *Panax ginseng* C.A. meyer is commonly referred to as Chinese of Korean ginseng and can be found in the mountainous forest of Eastern Asia (Bahrke and Morgan, 1994). *Panax quinquefolius*, known as North American ginseng, is grown in Canada and the United States (Bahrke and Morgan, 1994). Neither Russian ginseng (*Eleutherococcus senticosus*) nor Brazilian ginseng (*Pfaffia paniculata*) contain the ginsenosides characteristic of the *Panax* species (Vigano and Ceppi, 1994; Bahrke and Morgan, 1994). Ginseng is a perennial which grows naturally in mixed hardwood forests, preferring shade and well-drained soil (Goldstein, 1975). It takes approximately 5 years from the time of germination for the plant to reach maturity (Goldstein, 1975); ginseng is generally harvested after growing for four to seven years (Bahrke and Morgan, 1994; Liu and Xiao, 1992). Mature ginseng plants reach an average height of 8 to 15 inches, flower from June to July, and have vermilion berries from approximately July to the first frost (Goldstein, 1975). The roots of the mature

plants are typically 2 to 6 inches in length with a bifurcate shape (Goldstein, 1975). The entire ginseng plant contains pharmacologically active chemicals, however the root is considered to be the most valuable component (Bahrke and Morgan, 1994; Goldstein, 1975; Liu and Xiao, 1992).

The main active constituents of the *Panax* species are the triterpenoid glycosides of the dammaran series, which can further be divided into three types: oleanolic acid, Panaxadiol, and Panaxatriol types (Bahrke and Morgan, 1994; Liu and Xiao, 1992). These constituents are most commonly referred to as ginsenosides, but are also known as saponins (Bahrke and Morgan, 1994; Liu and Xiao, 1992). The ginsenosides are named Rx, where x is a, b1, b2, c, d, e, f, g1, g2, g3, h1, h2, or o, depending on their position on thin layer chromatograms (Bahrke and Morgan, 1994). Over 28 different ginsenosides have been identified (Liu and Xiao, 1992). Chinese ginseng is characterized by an abundance of the ginsenosides Rc and Rg1 (Bahrke and Morgan, 1994), while North American ginseng is characterized by the absence of Rf and a higher Rb1 to Rb2 ratio than Chinese ginseng (Kitts et al., 2000). The ginsenoside content of the plants varies with age, season of harvest, and method of preservation (Bahrke and Morgan, 1994). For example, the ginsenosides Ro, Rb, and Rg in Chinese ginseng are lowest at 2 years of growth, highest at 4 or 5 years of growth, and decrease thereafter (Liu and Xiao, 1992). Other constituents of ginseng include: polysaccharides, flavonoids, daucosterin, mucilaginous substances, amino acids, bitter substances, vitamins, choline, pectin, and fatty and ethereal oils (Liu and Xiao, 1992). Little information is available on the

chemical or pharmacological properties of these minor constituents or on their impact on the human immune system.

3. Physiologic Effects of Ginseng

It has been proposed that ginseng consumption has both general effects on the whole body and specific effects on various organ systems. An adaptogen is a substance which causes a state of “non-specifically increased resistance” (Bahrke and Morgan, 1994). Adaptogens are considered to be generally innocuous and have the following characteristics: increase resistance against stressful physical, chemical, or biological influences, lack specificity of action, and have a normalizing effect (Bahrke and Morgan, 1994). For example, an adaptogen could regulate both hypertension and hypotension. Proposed general adaptogenic effects of ginseng include analgesia, anticonvulsive effects, improved stress control, and improved learning and long-term memory (Bahrke and Morgan, 1994). It is also reported to reduce fatigue and improve endurance (Wong et al., 1998). However, few of these effects have been well investigated, perhaps due to difficulties associated with quantifying benefits such as “quality of life” or “vitality” (O’Hara et al., 1998).

Ginseng is also reported to have specific effects on the cardiovascular, nervous, and immune systems in both humans and animals. North American ginseng extract (NAGE) has been shown to chelate transition ion metals and scavenge free radicals, thereby acting as an antioxidant (Kitts et al., 2000). This antioxidant ability is maintained in both lipid-soluble and water-soluble conditions (Kitts et al., 2000). NAGE significantly reduced

lipid peroxidation induced by transition metals or a haemoglobin catalyst, inhibited non site-specific DNA strand breakage and suppressed protein oxidation induced by Fenton agents (Kitts et al., 2000).

4. Effects of Ginseng on Immune Function

The effects of ginseng consumption on immune function have been studied under a variety of conditions in both animals and humans. The majority of these studies have been conducted using *Panax ginseng* C.A. Meyer.

Scaglione et al. (1990) examined the effects of *Panax ginseng* extract G115 and an aqueous extract on immune parameters in healthy adult volunteers aged 18 – 50 years. The double-blind study administered 200 mg of either G115 or aqueous extract per day for 8 weeks (n = 20 per group). A third group (n = 20) received placebo capsules for the duration of the study. Venous blood samples were collected at the beginning of the study and after 4 and 8 weeks of treatment. Chemotaxis of circulating polymorphonuclear lymphocytes (PMN) increased significantly after 4 and 8 weeks of G115 and aqueous extract treatment, but not after placebo treatment. Phagocytosis of microorganisms by PMN increased after 4 and 8 weeks of G115 treatment and after 8 weeks of aqueous extract treatment. Total T-lymphocytes (CD3+) increased after 4 and 8 weeks of treatment with either ginseng preparation. T-helper subsets (CD4+) increased after 8 weeks of aqueous extract treatment and after 4 and 8 weeks of G115 treatment; no significant increases were seen in the T-cytotoxic (CD8+) lymphocyte subset. The T-helper/T-cytotoxic ratio was increased after 4 and 8 weeks of G115 treatment.

Lymphocyte proliferation in response to stimulation by concanavalin A (Con A), pokeweed mitogen (PWM) and lipopolysaccharide (LPS) increased after 4 and 8 weeks of G115 treatment; aqueous extract treatment increased proliferation after 8 weeks of administration in Con A and PWM-stimulated lymphocytes. Natural killer cell activity was increased after 8 weeks of aqueous extract administration and 4 and 8 weeks of G115 administration. While both ginseng extracts were effective at stimulating immune function in both males and females, the proprietary extract G115 was most effective.

In another double-blind, placebo-controlled trial, 300 mg of *Panax ginseng* was administered daily to healthy young Thai males (ages 21 – 22; n = 10 per group) for eight weeks (Srisurapanon et al., 1997). No initial differences in total and differential leukocyte counts or lymphocyte subsets were detected between the placebo and ginseng-treated groups. Ginseng treatment resulted in a significant decrease in circulating neutrophils and monocytes after 4 and 8 weeks of administration; total leukocyte count decreased only after 8 weeks of ginseng administration. No significant differences in lymphocyte subtypes were detected after ginseng administration. The authors concluded that since detected changes in circulating neutrophils and monocytes were small and no other changes occurred in lymphocyte subsets, administration of 300 mg per day of *Panax ginseng* extract for 8 weeks was not immunostimulatory (Srisurapanon et al., 1997).

Mizuno et al. (1994) investigated the effects of both wild and cultured *Panax ginseng* extracts on the proliferation of murine splenocytes *in vitro*. Wild ginseng was found to

have a greater concentration of the ginsenosides Rg1, Re, and Rd and fewer of the ginsenosides Rc, Rb1, and Rb2 than cultured ginseng. Wild *Panax ginseng* had the same mitogenic activity as Con A, whereas cultured *Panax ginseng* did not stimulate proliferation of splenocytes above control levels. Mizuno et al. (1994) also administered both ginseng extracts *in vivo* (200 mg/kg mouse weight) for 30 days. Splenocytes were then isolated and lymphocyte subsets analyzed via flow cytometry. Wild ginseng extract was also shown to increase the percentages of pan T-cells(CD3+), helper T-cells (CD4+), and cytotoxic T-cells (CD8+) above control levels. No effect of cultured ginseng extract was found on lymphocyte subsets. Thus it appears that the ginsenosides Rg1, Re, or Rd are important for the alteration of lymphocyte subsets and lymphocyte proliferation. It is also possible that the extract of wild *Panax ginseng* contained different polysaccharides than the cultured *Panax ginseng* which may be important for ginseng's effects on the immune system.

Intraperitoneal administration of *Panax ginseng* Rg1 (10 mg/kg) to mice for three consecutive days prior to infection with sheep red blood cells was performed by Kenarova et al. (1990) in order to determine the effects of Rg1 on the immune response. The plaque forming cell number was increased in the Rg1 treated mice compared to control mice 5, 7, and 10 days after infection. Rg1 treatment increased the number of antigen-reactive T cells 3, 5, and 10 days after infection; T-helper cell numbers were also increased after Rg1 treatment. Natural killer cell activity against chick erythrocytes was increased after Rg1 treatment for up to 10 days after infection. *In vitro* IL-1 production by peritoneal macrophages was increased when the cells were incubated directly with

Rg1; this effect was greater when cells were treated with 25 μg/ml Rg1 than with 50 μg/ml Rg1. Thus ginsenoside Rg1 modulated both humoral and cell-mediated immunity in mice. It is possible that low doses of Rg1 are immunostimulatory while high doses are immunosuppressive.

Panax ginseng extract administered with 6-MFA (an interferon-inducing antiviral substance) has also been shown to synergistically enhance protection of mice from viral infection, compared to 6-MFA treatment alone (Singh et al., 1983; Singh et al., 1984). In humans, *Panax ginseng* extract administered in combination with anti-influenza vaccine significantly reduced the incidence of influenza and the common cold (Scaglione et al., 1996). In this placebo-controlled trial, participants received 100 mg *Panax ginseng* extract daily for 12 weeks. Natural killer cell activity and antibody titers were significantly increased with ginseng administration compared to placebo treatment after both 8 and 12 weeks.

Borchers et al. (1998) isolated peripheral lymphocytes from 20 healthy volunteers (8 females and 12 males; aged 20 – 50 years) in order to determine the effects of different types, brands, and concentration of ginseng on in vitro proliferation. American ginseng increased proliferation at dilutions ranging from 10^{-7} to 10^{-2}; *Panax ginseng* had no effect on proliferation at any dilution. No effect of American ginseng or *Panax ginseng* on cell viability were found. When lymphocyte proliferation was compared within individuals, *Panax ginseng* was found to be cytotoxic at higher concentrations. The

authors conclude that ginseng can alter lymphocyte proliferation, is potentially cytostatic or cytotoxic, and biological effects vary with species, brand, and concentration.

Ginsenoside Rg1 has been shown to increase *in vitro* nitric oxide (NO) production in macrophages activated by IFN-γ; unstimulated macrophages did not respond to Rg1 (Fan et al., 1995). Rg1 also increased nitric oxide synthase (NOS) activity in activated macrophages (Fan et al., 1995). No significant effects of ginsenoside Rb1 were found on NO production or NOS activity on macrophages (Fan et al., 1995). Rg1 increased tumor cell killing by activated macrophages (Fan et al., 1995).

It thus appears that the various types of ginseng possess some immunomodulatory activity. However, the mechanisms of action of the various ginsenosides on the many cells of the immune system remain unclear. It is particularly unclear as to whether this immunomodulation is of clinical significance in relatively healthy individuals or under times of stress. Many studies regarding the effects of ginseng on the immune system were performed on cultured cells and the physiological relevance of these studies is uncertain. By implementing an exercise stress protocol, the present study is designed to further clarify the effects of North American ginseng on the immune function of healthy young men.

Chapter Three

Experimental Design and Methodology

A. Experimental Design

The present study was a randomized, placebo-controlled cross-over design. Subjects were randomized, half receiving ginseng and half receiving placebo, for a one month period. An exercise stress protocol and blood collection were performed at the end of the one month treatment period. A three-month washout period followed the blood collection, after which subjects received the opposite treatment for one month. All research was conducted at the University of Alberta. Ethical approval for the study was obtained from the Human Ethics Review Committee of the Faculty of Agriculture, Forestry, and Home Economics.

It was estimated that a sample size of 10 subjects was required. Given that we expected some attrition, 14 subjects were enrolled in the study. Participation in the study was based on the following eligibility criteria: male; aged 18 – 35 years; free of illness which would inhibit their ability to participate in the exercise protocol; free of diabetes, thyroid, renal, or autoimmune disease; not taking any medication or herbal supplements; non-smoking. Healthy men were recruited via posters on the University of Alberta campus (Appendix A), a similar student newspaper advertisement, and an on-line graduate student newsletter. Interested volunteers were screened via telephone or email to meet the recruitment criteria. Those individuals who met the recruitment criteria were asked to complete a demographic questionnaire (Appendix B) and undergo VO_{2max}

testing. Individuals with a VO_{2max} less than 50 ml/kg/min and a body mass index of 20 – 27 were asked to participate in the study. Participants were provided with an information package and provided consent to study participation (Appendix C).

B. Methodology

1. Aerobic Fitness Assessment (VO_{2max}):

Aerobic fitness level was measured by a maximal exertion VO_{2max} test on a Monark 818E cycle ergometer (Varberg, Sweden); respiratory gases were continuously monitored and averaged every 15 seconds on an automated metabolic measurement system (D-series Gas Exchange System, MedGraphics, CA) in the Women's Laboratory (E455) of the Van Vliet Centre. This test determines maximal aerobic exercise power by measuring oxygen uptake under maximal exertion conditions. It was performed in order to determine the correct work load for each individual during the exercise stress protocol. Prior to testing, participants were required to complete a Physical Activity and Readiness Questionnaire (PAR-Q; Appendix D) to screen for possible medical complications that would prevent the individual from performing the test. The test involved a progressive increase in exercise intensity to the point where the participant would no longer continue to exercise. Participants started pedalling at a resistance of 1.0 kiloponds (kp) at a frequency of pedaling between 60 and 70 revolutions per minute; every two minutes, the resistance was increased by 0.5 kp until ventilatory threshold (VT) was attained, after which resistance was increased by 0.5 kp every minute until VO_{2max} was attained. VT was defined as a decrease and plateau in VE/VCO_2 prior to a systematic increase with increased power output as well as a respiratory exchange ratio (RER) greater than 1.05

(Bhambhani and Singh, 1985). The criteria defined for attainment of VO_{2max} were (a) a levelling or decrease in VO_2 with increasing work, (b) a plateau in heart rate, (c) respiratory exchange ratio (RER) > 1.1 and, (d) volitional fatigue (Thoden, 1991). Although the test required the participant to exercise to exhaustion, he was ultimately in control of terminating the test. Heart rate (Polar Electro Heart Rate Monitor, Polar USA Inc., Stanford, CN) was monitored every minute. Muscle discomfort/soreness, shortness of breath, and abnormal heart beat and blood pressure are possible side effects associated with maximal aerobic exertion, but are rare in healthy young adults.

2. Ginseng and Placebo Treatment

North American whole root ginseng extract powder capsules were obtained from Chai-Na-Ta Corp., Ltd. (Langley, B.C., Canada). All capsules utilized in the study were from the same lot number. Both ginseng and placebo capsules were solid-white two-piece capsules made out of gelatin from animal sources and were identical in size and colour. Placebo capsules contained 350 mg of corn starch per capsule.

Participants received a total of 1125 mg of extract daily. Participants were instructed to take one capsule 30 minutes prior to eating breakfast, lunch, and dinner for a period of 35 days. In addition, participants were provided with a detailed set of written take-home instructions regarding capsule intake and storage (Appendix E). Participants were

randomly assigned to receive either ginseng or placebo for the first treatment period and received the opposite treatment for the second treatment period. Participants were not informed as to whether they were receiving ginseng or placebo capsules. All participants were advised about the potential side effects of ginseng consumption (Appendix F) and were asked to contact the researchers immediately should any side-effects as a result of treatment arise. In addition, participants were contacted frequently throughout the treatment periods in order to record side-effects, if any (Appendix F).

At the completion of each one-month treatment phase, volunteers were asked to undergo the following procedures:

3. Assessment of Dietary Intakes:

Dietary intakes were assessed by three-day food records (Appendix G). Each participant was instructed by a dietetic intern on how to record food intake, with particular detail in regard to serving size, amount, brand name, and method of cooking. Emphasis was placed on maintaining typical eating habits throughout the three-day assessment period. The food records were performed by each participant for two week-days and one weekend day around the time of the exercise stress protocol. Once completed, food records were reviewed by the dietetic intern and confirmed for accuracy with the participant. The records were analyzed by a computerized nutrient analysis software program, Food Processor II for Windows (Food Processor II™ for Windows, ESHA Research, Portland, Oregon). The same dietetic intern reviewed and analyzed all of the food records. Macronutrient ratios and total energy intake in kilocalories were

determined from the computerized analysis in order to compare dietary patterns between the two treatment periods.

4. Self-Report Activity Records:

On the day of the exercise stress protocol, participants were asked to complete the Modified Baecke Questionnaire on Physical Activity (Pols et al., 1995; Appendix H). The Baecke questionnaire provides an index of the amount of activity spend during work, sports, and leisure time.

5. Hydrostatic (Underwater) Weighing:

All measurements were performed in E455 of the Van Vliet Centre. The helium dilution technique (Motley, 1957) was used to determine participants' residual lung volumes (RV) prior to the underwater weighing procedure. Each participant was required to inhale a minute amount of inert helium gas and perform a series of breathing exercises into a SensorMedics 2450 Pulmonary Function Cart (Yorba Linda, CA). Vital capacity and expiratory reserve volume were measured and residual volume was calculated.

Body weight wearing a bathing suit was measured to the nearest 0.1 kg on a pre-calibrated balance-beam scale (Healthometer, Continental Scale Corporation, Bridgeview, IL). Height without shoes was measured after a full inspiration and recorded to the nearest 0.1 cm using a measuring tape. Underwater weight was measured with a computerized strain-gauge system and corrected for functional residual volume. The

(collected in EDTA-coated vacutainers) was used for complete blood count and differential analysis. Blood drawn at other time points was used to assess stress hormone response (reported by Humphreys, 2001). A mouthpiece and hose system was attached to the individual for the first six minutes of exercise and at various intervals throughout the remaining 30 minutes of the exercise protocol to monitor substrate oxidation and respiratory exchange ratio. Participants were encouraged to drink water throughout the protocol, but no other food or drink was permitted.

The following procedures were performed on blood samples collected pre-exercise (-15 minutes), post-exercise (36 minutes) and at recovery (66 minutes):

7. Hematological Analysis

Three ml of whole blood (collected in EDTA-coated vacutainers) was analyzed for complete blood count, using a Coulter STKS instrument (Coulter Electronics Inc, Hialeh, FL) and manual differential. Analysis was performed by the staff at Dynacare-Kasper Laboratories (Edmonton, AB).

8. Mononuclear Cell Phenotyping

Lymphocyte subsets were characterized by immunofluorescence assay using monoclonal antibodies specific for the different human mononuclear cell subsets. Antibodies and the lymphocyte populations they recognize are indicated in Table 1. A 96-well, v-bottom, nonsterile microtiter plate (Fisher, Edmonton, AB) was conditioned for at least 15 minutes with 4 % (v/v) fetal calf serum (FCS; Gibco BRL, Grand Island,

participant wore a bathing suit in the temperature-regulated (34-37°C) water pool. Multiple trials were performed until the difference in body density between trials was less than 0.005; these three trials were then averaged to obtain final values for body density. Percentage body fat, fat mass, and fat-free mass were estimated from body density using the equation of Siri (1956). The results of body composition determination are presented for descriptive purposes only; these results are discussed in greater detail by Humphreys (2001).

6. Exercise Stress Protocol and Blood Collection

The exercise stress protocol and blood collection were performed in the Women's Health and Physical Activity Lab (E-455) at either 2:00 pm or 3:30 pm in order to control for the diurnal variation in serum cortisol. Participants were asked to refrain from exercising the day of the protocol and to refrain from eating for two hours prior to the protocol. Participants performed 36 minutes of continuous exercise comprised of 6 minute warm-up at 1.5 kp and 50 rpm, 15 minutes at 80% ventilatory threshold (60 to 70 rpm), and 15 minutes at 100% ventilatory threshold (60 to 70 rpm) on a stationary bicycle. Ventilatory threshold was determined from the peak VO_{2max} for each participant. An intravenous catheter was inserted into the forearm prior to the start of the exercise test and venous blood samples were collected serially before (-15, 0 minutes), during (21 minutes), and after exercise (36, 51, 66, 96 minutes). A 13 ml sample of blood was drawn at times –15, 36, and 66 minutes for immunological analysis; 10 ml of blood (collected in heparinized vacutainers) was used to determine mononuclear cell phenotypes, neutrophil function, and lymphocyte proliferation. Three ml of blood

NY) in phosphate buffered saline (PBS). Whole blood (100 μL) was added to each well with 100 μL warm lysis buffer at room temperature for up to 5 minutes. The plate was then centrifuged at 1000 rpm for 3 minutes at 4°C (Beckman J2-HC, Beckman Instruments, Palo Alto, CA) to pellet cells. Supernatant was removed with a small gauge needle, leaving the pellet of cells undisturbed. Red blood cell lysis was repeated using 200 μL warmed lysis buffer. The plate was again centrifuged at 1000 rpm for 3 minutes at 4°C. Samples were washed twice with cold 4% FCS in PBS (v/v) and centrifuged at 1000 rpm for 5 minutes at 4°C. Monoclonal antibodies (mAb) were added to each well and incubated at 4°C for 30 minutes. Samples were washed twice with cold 4% FCS in PBS (v/v) and centrifuged at 1000 rpm for 5 minutes at 4°C. Finally, 200 μL of PBS + 2% (w/v) paraformaldehyde (Anachemia Science, Montreal, PQ) was added to each well to fix cells. Plates were wrapped in foil and refrigerated. All samples were acquired and analyzed within 72 hours on a FACScan (Becton-Dickenson, Sunnyvale, CA) according to relative fluorescence intensity using CellQuest software (Becton-Dickenson). Lymphocytes stimulated by phytohemaglutinin (PHA) and cultured for 48 hours as described below were phenotyped in a similar manner.

9. Isolation of Peripheral Mononuclear Cells

Peripheral mononuclear cells were isolated under sterile conditions. One ml of heparinized whole blood was layered on top of 3 ml of room temperature Histopaque-1077 (Sigma Chemical Co., St. Louis, MO) and centrifuged in biohazardous containers at 1800 rpm for 40 minutes at room temperature (21°C) without a brake. Plasma was collected and immediately frozen at –70°C in sterile plastic tubes. Cells at the opaque

interface of histopaque and plasma were collected and transferred to sterile 15 ml conical tubes. Isolated mononuclear cells were then washed with 2% (v/v) bovine serum albumin (BSA Fraction V; Sigma Chemical, St. Louis, MO) in PBS and centrifuged at 1500 rpm for 10 minutes at 4°C. The supernatant was discarded and the cells resuspended with complete culture media (CCM; RPMI 1640; Gibco BRL, Grand Island, NY), 4% (v/v) FCS and 1% (v/v) antibiotic/antimycotic (Gibco, Burlington, ON). Cell viability was assessed using trypan blue exclusion (Sigma Chemicals, St. Louis, MO). Mononuclear cells were used for cytokine and lymphocyte proliferation assays.

10. Mitogenic Stimulation of Peripheral Mononuclear Cells

Peripheral mononuclear cells isolated above were seeded in triplicate into sterile 96-well tissue-culture-treated, round–bottom plates (200 μL of 1.0 x 10^6 cells/ml per well; Corning, New York, NY). Phytohemaglutinin (5 ng/L; PHA; ICN, Montreal, PQ) was added as a mitogen to wells. CCM (20 μL) was added to background wells to determine unstimulated proliferation. Plates were incubated for 30 and 54 hours at 37°C and 5% CO_2. Cells were then pulsed with 1 μCi 3H-thymidine (Amersham, ON) and incubated for another 18 hours. At 48 and 72 hours, cells were harvested onto glass fibre filter mats (Skatron, Suffolk, UK) with a multiwell harvester (Skatron, Lier, Norway). Samples were transferred to scintillation vials containing Ecolite® scintillation fluid (ICN, Montreal, PQ) and counted on a beta counter (Beckman LS 5801, Beckman Instruments, Missisauga, ON). The total decays per minute (dpm) for each well were used to determine thymidine incorporation due to cellular proliferation. Stimulation indices (SI) were calculated using the formula:

$$SI = \frac{(\text{[3H] thymidine (dpm) incorporated by stimulated cells}) - (\text{[3H] thymidine (dpm) incorporated by unstimulated cells})}{(\text{[3H] thymidine (dpm) incorporated by unstimulated cells})}$$

11. Lymphocyte Interleukin-2 (IL-2) Production

Four ml of peripheral mononuclear cells isolated above were prepared at a concentration of 1.0 x 10^6 cells/ml in CCM; 1 ml of cell suspension was then placed in a sterile plastic 5 ml tube. PHA (100µL) was added in the same concentration (5 ηg/L) as for the mitogen stimulation assay. The same volume of CCM was added to separate tubes to determine unstimulated cytokine production. Tubes were covered and incubated for 48 hours at 37°C with 5% CO_2. Cells were then pelleted by centrifuging at 1500 rpm for 10 minutes. The supernatant was transferred to 1 ml sterile plastic tubes and frozen at –70°C for subsequent cytokine analysis. Cells stimulated by PHA were removed and phenotyped as described above.

Supernatants were thawed and used to determine IL-2 production via a colorimetric enzyme-linked immunosorbent assay (ELISA). An OptEIA™ set for human IL-2 containing all antibodies and standards was purchased from Pharmingen, San Diego, CA. Microwells (Immulon® 2 HB high binding flat bottom microtiter plates, Dynex Technologies Inc, Chantilly, VA) were coated with 100 µL anti-IL-2 capture antibody, sealed, and incubated overnight at 4°C. Wells were washed the following day with PBS/Tween 3 times and blotted on absorbent paper. Blocking buffer (200 µL per well)

was added and incubated at room temperature for 30 minutes in order to prevent non-specific binding. Plates were washed 3 times with PBS/Tween. Standards (7.8 ρg/ml to 500 ρg/ml) were prepared according to instructions and 100 μL added to each well. PHA-stimulated samples were added and diluted as required with blocking buffer/Tween. Plates were sealed and incubated at room temperature for 2 hours and subsequently washed 5 times with PBS/Tween. Biotinylated anti-IL-2 detecting antibody (working detector; 2.0 μg/ml) was added to each well and incubated for 1 hour. Plates were then washed 7 times with PBS/Tween. 100 μL of avidin-horseradish peroxidase (substrate solution; 0.5 μL/ml) was added to each well and incubated for 30 minutes in the dark at room temperature. Finally 50 μL of 2N H_2SO_4 (stopping solution) was added to each well. Optical density was read immediately at 450 ηm on a plate reader (Model EL309; Bio-tek Instruments Inc., Burlington, VT).

12. Neutrophil Oxidative Burst

Three hundred μL of whole blood was lysed with 4 mL lysis buffer at 37°C for 5 minutes in a 5 mL polypropylene tube. The sample was pelleted by centrifuging at 1500 rpm for 5 minutes and lysis was repeated if necessary. The pellet of cells was washed by resuspending in 4 mL of 1% (v/v) bovine serum albumin (BSA, Fraction V; Sigma Chemicals, St. Louis, MO) in PBS and centrifuged at 1500 rpm for 5 minutes. The pellet was resuspended in 400 μL buffer and incubated with 1.8 μL dihydrorhodamine 123 (DHR; Molecular Probes, Eugene, OR) for 5 minutes at 37°C. Background oxidative burst was measured by removing 100 μL of the cell suspension and placing on ice (time 0 minutes). A phorbol myristate acetate solution (3.2×10^3 nM; PMA; ICN, Montreal,

PQ) was added to the remaining 300 μL cell suspension in the reaction tube. Finally, 100 μL of cell suspension was removed 5, 10, and 15 minutes after the addition of PMA to the reaction tube. The sample was placed on ice at each time point to prevent any further reaction. The oxidation of DHR to rhodamine 123 was immediately quantified using a 488 ηm line for excitation and a 525 ηm line for collection using flow cytometry (FACScan, Becton Dickenson, San Jose, CA) and analyzed with CellQuest software (Becton Dickenson). Neutrophils were differentiated from remaining leukocytes and debris by combined measures of forward and side light scatter characteristics. Mean channel fluorescence of gated neutrophils at each time point was used to estimate the oxidative burst. The change in oxidative burst after stimulation with PMA was determined using the ratio given by the formula (5, 10, or 15 minute value ÷ 0 minute value).

C. Statistical Analysis

Paired, dependent t-tests were used to analyze aerobic fitness, dietary intakes, physical activity indices, and body composition between ginseng and placebo treatments. All immunology data were analyzed by two-way repeated measure analysis of variance (ANOVA). The statistical significance level was set at $p < 0.05$. When differences due to treatment were detected by the repeated measures analysis, lsmeans were used to determine which of the pre-exercise, post-exercise and recovery time points were different between ginseng and placebo groups. When differences due to time (i.e.: the effect of exercise) were detected by the repeated measures analysis, paired t-tests were used to determine the differences between pre-exercise and post-exercise and pre-

exercise and recovery time periods within each treatment group. All statistical analyses were conducted using the software program SAS (version 8.1, SAS Institute, Cary, NC).

Monoclonal Antibody	**Isotype**	**Lymphocyte Population**	**Supplied By**
CD-3	IgG2a	TCR receptor; pan-T lymphocyte	Serotec[a]
CD-20	IgG2a	Pan B-lymphocyte	Serotec[a]
CD-16	IgG1	FcγIII receptor; NK cells, mature monocytes	BD PharMingen[b]
CD-4	IgG1	Helper T-lymphocyte	Sigma Chemicals[c]
CD-8	IgG2a	Cytotoxic T-lymphocyte	Sigma Chemicals[c]
CD-45RO	IgG2a	LCA for antigen memory on T and B lymphocytes	Serotec[a]
CD-45RA	IgG2a	LCA for antigen naivity on T and B lymphocytes	Serotec[a]

[a] Serotec, Raleigh, NC

[b] BD PharMingen, San Diego, CA

[c] Sigma Chemicals, St. Louis, MO

Table 1: Specificities of monoclonal anitbodies used to phenotype peripheral blood mononuclear cells. All monoclonal antibodies were purchased prelabelled from the indicated suppliers.

Abbreviations: CD=cluster of differentiation; IgG=immunoglobulin; TCR=T-cell receptor; NK=natural killer cell; LCA=leukocyte common antigen; IL-2=interleukin-2

Chapter Four

Results

A. Participant Characteristics

Of the fourteen males who enrolled in the study, ten completed the study and four participants withdrew for personal reasons. All participants reported to be in good health, prior to, and throughout the study. There were no significant differences in body composition, VO_{2max}, VT, energy intake, or physical activity indices between the ginseng and placebo treatments (Tables 2 – 5). Dietary analysis from three-day food records was performed on eight subjects (two records were unavailable in the second phase of the study). These data are presented for descriptive purposes only.

B. Side Effects of Treatment

No participants reported taking any prescription medications or herbal supplements at any time during the study, other than the assigned treatment. Two subjects reported mild insomnia and hot flashes during the first few days of ginseng treatment; these side-effects subsided after a week of treatment. No other adverse side effects were reported during either the ginseng or placebo treatment periods or as a result of the exercise stress protocol.

C. Immune Parameters

1. Hematological Analysis

The complete blood count and differential was obtained for all subjects during both treatment periods (n = 10). The total white blood cell (WBC) count at rest was not different between ginseng and placebo treatment periods ($5.62 \pm 0.79 \times 10^9$ vs $5.91 \pm 1.55 \times 10^9$, respectively; Table 6). Total WBC counts were significantly altered due to exercise, with an increase in WBC post-exercise in both treatment groups ($p=0.0001$; Table 6). Further analysis revealed that post-exercise WBC counts were significantly elevated above pre-exercise levels for both groups, and that recovery WBC counts were not different from pre-exercise levels (Table 6). There were no significant effects of ginseng versus placebo treatment on the number of WBC. White blood cell counts remained within reference ranges ($4\text{-}11 \times 10^9$ cells/litre) during all three time points and for both treatment groups.

Neutrophil concentrations were not significantly different between ginseng and placebo groups prior to the onset of exercise ($3.08 \pm 0.86 \times 10^9$ vs. $3.31 \pm 1.10 \times 10^9$, respectively; Table 7). Circulating neutrophil numbers were significantly altered due to exercise ($p=0.0001$; Table 7); post-exercise neutrophil numbers were increased above pre-exercise values in both groups. Treatment with either ginseng or placebo did not significantly alter the number of circulating neutrophils. Neutrophil counts were within the reference range ($1.8\text{-}7.5 \times 10^9$ cells/litre) for both treatments and all time periods. The proportion of circulating neutrophils was significantly altered by exercise ($p=0.0016$;

Table 7); significant differences within each treatment group were not identified. There was no effect of treatment on the proportion of circulating neutrophils.

Circulating lymphocyte numbers were significantly altered by exercise ($p=0.0001$; Table 8). Post-exercise lymphocyte concentrations were significantly increased in both groups compared to resting values (Table 8). The overall pattern of change of lymphocyte concentrations was not significantly altered due to ginseng or placebo treatment. Lymphocytes were within the reference range ($1 - 4 \times 10^9$ cells/litre) for all times and both treatment periods. The proportion of circulating lymphocytes was significantly altered by exercise, but not by treatment (Table 8). Within each treatment group, no significant differences in the proportion of circulating lymphocytes could be identified.

The concentration of circulating monocytes was significantly altered by exercise, but not by treatment (Table 9). Within the ginseng-treated group, the number of monocytes increased significantly post-exercise ($p<0.05$; Table 9). There were no effects of exercise on the number of circulating monocytes in the placebo-treated group. The proportion of circulating monocytes was not significantly altered by either exercise or treatment (Table 9).

2. Mononuclear Cell Phenotypes

The effects of treatment and exercise on lymphocyte subsets are presented in Tables 10, 11, and 12. Lymphocyte subsets were determined using prelabelled

monoclonal antibodies for specific cell surface markers and flow cytometry. There were no significant effects of treatment on either the percent or number of mononuclear cell phenotypes. The percent of CD3+ (pan-T-cells) was significantly altered by exercise ($p = 0.014$; Table 10); similarly, the number of CD3+ cells was altered by exercise ($p = 0.0002$; Table 11). Further analysis to determine which time points were affected by exercise revealed that in the placebo group, the post-exercise percent of CD3+ cells was significantly reduced compared to pre-exercise ($p<0.05$) and that the recovery expression of CD3 had returned to pre-exercise values (Table 10). However, the absolute number of pan-T-cells was increased in the post-exercise period for the placebo-treated group (Table 11). In the ginseng treatment group, there were no significant effects of exercise on the percent or number of CD3+ cells.

There were no effects of treatment on the percent or number of CD4+ cells, as determined by the repeated measures analysis. Exercise had a significant impact ($p=0.0003$) on the percent of CD4+ cells; this difference was found to be due to a reduction in the proportion of CD4+ cells post-exercise in the placebo group ($p<0.05$; Table 10). The absolute number of CD4+ cells was also altered by exercise; the number of T-helper lymphocytes was significantly reduced in the placebo-treated group at recovery ($p<0.05$; Table 11). There were no effects of either treatment or exercise on the percent of CD8+ (T-cytotoxic) cells, as determined by repeated measures analysis (Table 10). The absolute number of CD8+ cells was significantly increased post-exercise in the placebo group ($p<0.05$; Table 11). The CD4/CD8 ratio was not significantly altered by exercise or by treatment (Table 10). There were no differences due treatment in the

percent of CD4+/CD45RO+, CD4+/CD45RA+, or the CD4+ RO/RA ratio (Table 12). There was a significant increase in the expression of CD8+/CD45RO+ cells in the ginseng group compared to the placebo group (Table 12); there were no differences in the percent of CD8+/CD45RA+ or the CD8+ RO/RA ratio.

The percent of CD20+ cells (B-cells; Table 10) was significantly altered by exercise ($p=0.0001$), but not by treatment as determined by repeated measures. Analysis determined that post-exercise,the relative percent of CD20+ cells was significantly reduced ($p<0.05$) in the ginseng treatment group (Table 10). In the placebo group, the percent of CD20+ lymphocytes was significantly reduced post-exercise and significantly increased at recovery compared to pre-exercise levels. The absolute number of CD20+ cells was not significantly altered by treatment (Table 11). Exercise significantly altered the number of CD20+ cells ($p= 0.0015$; Table 11); this effect was due to an increase in the absolute number of CD20+ cells in the placebo group at the post-exercise period. The percent and number of CD16+ cells was also significantly impacted by exercise, but not by treatment (Tables 10 and 11). The percent of CD16+ lymphocytes was significantly increased in the ginseng group post-exercise compared to pre-exercise ($p<0.05$; Table 10). The absolute number of CD16+ cells was increased post-exercise in both ginseng and placebo groups ($p<0.05$; Table 11).

3. Mitogenic Stimulation of Peripheral Mononuclear Cells

Unstimulated lymphocyte proliferation, as estimated by [^{3}H]-thymidine incorporation, was not significantly altered due to treatment (Table 13). After 48 hours, unstimulated lymphocytes in the ginseng group increased significantly in the recovery period compared to pre-exercise ($p<0.05$). After 72 hours, unstimulated lymphocytes in the ginseng group were also significantly increased in the recovery period ($p<0.05$). Treatment with either ginseng or placebo had no significant impact on the ability of lymphocytes to proliferation in response to PHA (Table 13). There were no effects of exercise on the ability of lymphocytes to proliferate in response to PHA after 48 hours (Table 13). After 72 hours, the ability of lyphocytes to proliferate in response to PHA was significantly altered by exercise ($p = 0.0261$; Table 13); further analysis did not reveal any significant differences in proliferation within each treatment group.

4. Lymphocyte Interleukin-2 (IL-2) Production

In vitro production of IL-2 was not detected in unstimulated lymphocytes. Lymphocytes stimulated by PHA for 48 hours produced IL-2 (Table 14). The pattern of IL-2 production was altered by ginseng versus placebo treatment ($p=0.0118$); no significant differences between treatments at each time point could be determined by lsmeans. It appears that ginseng increases IL-2 production post-exercise compared to placebo (Figure 5). Exercise had no significant impact on IL-2 production in either treatment group (Table 13).

5. Neutrophil Oxidative Burst

The oxidative burst activity before (0 minutes) and after (5, 10, 15 minutes) PMA stimulation was not significantly altered by either treatment of exercise (Table 15), as determined by the mean fluorescence of neutrophils on flow-cytometry.

Characteristic	**Ginseng (n = 10)**	**Placebo (n = 10)**
VO_{2max} (l/min)	3.3 ± 0.5 (2.4 – 3.9)	3.4 ± 0.4 (2.8 – 4.2)
VO_{2max} (ml/kg/min)	43.9 ± 5.8 (34.4 – 50.6)	45.0 ± 5.2 (38.0 – 51.3)
VT (l/min)	2.2 ± 0.5 (1.6 – 3.1)	2.2 ± 0.2 (1.8 – 2.6)
VT (ml/kg/min)	29.8 ± 5.6 (22.9 – 39.8)	29.6 ± 3.3 (21.7 – 33.2)
VT:%VO_{2max}	67.8 ± 7.3 (55.0 – 79.0)	66.0 ± 5.5 (56.0 – 73.0)
Max Heart Rate (bpm)	183 ± 11 (173.0 – 204.0)	182 ± 10 (170.0 – 199.0)

Table 2: Aerobic fitness between ginseng and placebo treatments. Data are reported as mean ± standard deviation and (range). Maximal oxygen uptake (VO_{2max}) and ventilatory threshold (VT) were determined under maximal exertion conditions by monitoring respiratory gases on an automated metabolic measurement system, as described in the methods. There were no significant differences in aerobic fitness between treatment groups.

Dietary Parameter	Ginseng (n = 8)	Placebo (n = 8)
Energy Intake (kcal/day)	2514 ± 291 (2137 – 2898)	2563 ± 500 (1955 – 3499)
Energy Intake (kcal/kg/day)	34 ± 6 (28 – 42)	35 ± 8 (26 – 48)
CHO Intake (g/day)	359 ± 41 (284 – 409)	377 ± 94 (278 – 546)
% CHO of Total Energy	57 ± 7 (47 – 70)	58 ± 6 (46 – 63)
Fat Intake (g/day)	78 ± 24 (47 – 117)	65 ± 15 (51 – 90)
% Fat of Total Energy	27 ± 6 (19 – 36)	23 ± 3 (18 – 27)
PRO Intake (g/day)	96 ± 18 (61 – 118)	106 ± 22 (83 – 144)
% PRO of Total Energy	16 ± 2 (11 – 18)	20 ± 4 (15 – 27)

Table 3: Total dietary energy, carbohydrate (CHO), fat, and protein (PRO) intakes between ginseng and placebo treatments. Data are reported as mean ± standard deviation and (range). Dietary analysis was performed on three-day food records taken during each treatment period. There were no significant differences in dietary intake parameters between treatment periods.

	Ginseng (n = 10)	**Placebo (n = 10)**
Physical Activity Index	7.57 ± 0.99 (6.30 – 9.10)	7.86 ± 1.13 (7.05 – 10.10)

Table 4: Self-reported physical activity index between ginseng and placebo treatments. Data are reported as mean ± standard deviation and (range). Physical activity during work, sports, and leisure time was assessed using the Modified Baecke Questionnaire (Pols et al., 1995). No significant differences existed between ginseng and placebo treatment periods.

Characteristic	**Ginseng (n = 10)**	**Placebo (n = 10)**
Weight (kg)	74.9 ± 4.8 (68.4 – 82.5)	75.6 ± 6.0 (66.6 – 84.6)
BMI (kg/m^2)	22.9 ± 1.3 (20.3 – 24.3)	23.1 ± 1.6 (19.9 – 24.6)
% Body Fat	15.6 ± 5.4 (10.1 – 26.0)	16.0 ± 5.9 (10.0 – 26.7)
Fat Mass (kg)	11.7 ± 4.1 (7.4 – 19.4)	12.2 ± 4.6 (7.4 – 19.7)
Fat Free Mass (kg)	63.2 ± 5.7 (55.0 – 69.4)	63.5 ± 6.4 (54.0 – 71.7)

Table 5: Body composition between ginseng and placebo treatment groups. Data are reported as mean ± standard deviation and (range). Body composition was determined by hydrostatic (underwater) weighing, as described in the methods. There were no significant differences in body composition between treatment periods.

	Pre-Exercise ($x10^9$cells)	Post-Exercise ($x10^9$cells)	Recovery ($x10^9$cells)	Effect of Time p-value	Effect of Time x Treatment p-value
Ginseng (n=10)	5.62 ± 0.79	7.43 ± 1.71 *	5.38 ± 1.71	p = 0.0001	p = 0.2650
Placebo (n=10)	5.91 ± 1.55	8.28 ± 1.74 *	6.32 ± 1.47		

Table 6: Effects of treatment and exercise on circulating white blood cell (WBC) counts. WBC counts were determined using a Coulter STKS instrument. Data are expressed as mean ± standard deviation. Significant differences due to exercise ($p=0.0001$) were determined using a repeated measures ANOVA. No significant differences between ginseng and placebo treatments were identified. Significant differences ($p<0.05$) from pre-exercise within the same group are indicated by (*).

	Treatment	Pre-Exercise	Post-Exercise	Recovery	Effect of Time p-value	Effect of Time x Treatment p-value
Neutrophil concentration ($x10^9$ cells/L)	**Ginseng**	3.08 ± 0.86	4.01 ± 1.17 *	3.16 ± 0.93	p = 0.0001	p = 0.4661
	Placebo	3.31 ± 1.10	4.39 ± 1.19 *	4.04 ± 1.34		
Neutrophil proportion (% of WBC)	**Ginseng**	54 ± 10	54 ± 7	58 ± 7	p = 0.0016	p = 0.2636
	Placebo	56 ± 9	53 ± 7	63 ± 7		

Table 7: Effects of treatment and exercise on circulating neutrophils. Neutrophil concentrations were determined using a Coulter STKS instrument and manual differential. Data are expressed as mean ± standard deviation. Significant differences due to exercise were determined using a repeated measures ANOVA. No significant differences between ginseng and placebo treatments were identified. Significant differences ($p<0.05$) from pre-exercise within the same group are indicated by (*).

	Treatment	**Pre-Exercise**	**Post-Exercise**	**Recovery**	**Effect of Time p-value**	**Effect of Time x Treatment p-value**
Lymphocyte concentration (x10^9 cells/L)	**Ginseng**	1.94 ± 0.52	2.59 ± 0.70 *	1.64 ± 0.37 *	p = 0.0001	p = 0.4630
	Placebo	1.93 ± 0.61	2.92 ± 0.64 *	1.66 ± 0.24		
Lymphocyte proportion (% of WBC)	**Ginseng**	35 ± 10	35 ± 5	30 ± 6	p = 0.0014	p = 0.3072
	Placebo	33 ± 6	36 ± 5	27 ± 6		

Table 8: Effects of treatment and exercise on circulating lymphocytes. Lymphocyte concentrations were determined using a Coulter STKS instrument and manual differential. Data are expressed as mean ± standard deviation. Significant differences due to exercise were determined using a repeated measures ANOVA. Significant differences ($p<0.05$) from pre-exercise within the same group are indicated by (*). No significant differences between ginseng and placebo treatments were identified.

	Treatment	**Pre-Exercise**	**Post-Exercise**	**Recovery**	**Effect of Time p-value**	**Effect of Time x Treatment p-value**
Monocyte concentration ($x10^9$ cells/L)	**Ginseng**	0.42 ± 0.15	0.73 ± 0.41 *	0.44 ± 0.13	p = 0.0034	p = 0.5749
	Placebo	0.53 ± 0.29	0.73 ± 0.17	0.45 ± 0.07		
Monocyte proportion (% of WBC)	**Ginseng**	8 ± 3	9 ± 4	9 ± 3	p = 0.0569	p = 0.6170
	Placebo	7 ± 2	9 ± 2	8 ± 1		

Table 9: Effects of treatment and exercise on circulating monocytes. Monocyte concentrations were determined using a Coulter STKS instrument and manual differential. Data are expressed as mean ± standard deviation. Significant differences due to exercise were determined using a repeated measures ANOVA. Significant differences ($p<0.05$) from pre-exercise within the same group are indicated by (*). No significant differences between ginseng and placebo treatments were identified.

Lymphocyte Subset	**Treatment**	**Pre-Exercise** (% of total gated cells)	**Post-Exercise** (% of total gated cells)	**Recovery** (% of total gated cells)	**Effect of Time p-value**	**Effect of Time x Treatment p-value**
CD3	Ginseng	74 ± 8	70 ± 8	75 ± 6	p = 0.0135	p = 0.7157
	Placebo	74 ± 8	68 ± 7 *	72 ± 15		
CD4	Ginseng	38 ± 13	33 ± 11	38 ± 12	p = 0.0003	p = 0.2425
	Placebo	37 ± 10	24 ± 7 *	29 ± 10		
CD8	Ginseng	30 ± 10	29 ± 9	35 ± 10	p = 0.1551	p = 0.3180
	Placebo	31 ± 11	30 ± 9	31 ± 8		
CD4/CD8 Ratio	Ginseng	1.6 ± 1.5	1.5 ± 1.2	1.2 ± 0.6	p = 0.0733	p = 0.1208
	Placebo	1.4 ± 0.8	0.9 ± 0.5	1.0 ± 0.5		
CD20	Ginseng	11 ± 3	10 ± 4 *	12 ± 4	p = 0.0001	p = 0.1682
	Placebo	11 ± 5	9 ± 3 *	12 ± 4 *		
CD16	Ginseng	24 ± 9	32 ± 7 *	22 ± 8	p = 0.0029	p = 0.4013
	Placebo	21 ± 12	27 ± 12	12 ± 8		

Table 10: Effects of exercise and ginseng on lymphocyte subsets of whole blood. Lymphocyte subsets were determined using prelabelled monoclonal antibodies for specific cell surface markers and flow cytometry. Data are expressed as relative percent means ± standard deviation (n=10). No significant differences existed in the pattern of change due to treatment as determined via repeated measures analysis and lsmeans. Within each group, significant differences from rest due to exercise ($p<0.05$; indicated by *) were determined via repeated measures analysis and paired t-tests.

Lymphocyte Subset	Treatment	Pre-Exercise (x 10^7 cells)	Post-Exercise (x 10^7 cells)	Recovery (x 10^7 cells)	Effect of Time p-value	Effect of Time x Treatment p-value
CD3	Ginseng	146 ± 50	182 ± 61	123 ± 28	p = 0.0002	p = 0.7151
	Placebo	133 ± 65	197 ± 45 *	120 ± 30		
CD4	Ginseng	75 ± 40	80 ± 34	64 ± 28	p = 0.0001	p = 0.3899
	Placebo	71 ± 24	70 ± 26	48 ± 19 *		
CD8	Ginseng	59 ± 55	76 ± 69	58 ± 54	p = 0.0010	p = 0.1170
	Placebo	63 ± 47	89 ± 37 *	50 ± 14		
CD20	Ginseng	21 ± 8	24 ± 9	20 ± 8	p = 0.0015	p = 0.2536
	Placebo	21 ± 10	27 ± 13 *	20 ± 9		
CD16	Ginseng	48 ± 20	89 ± 34 *	38 ± 17	p = 0.0010	p = 0.6038
	Placebo	38 ± 23	82 ± 48 *	19 ± 12		

Table 11: Effects of exercise and ginseng on lymphocyte subset concentrations of whole blood. Lymphocyte concentrations were calculated from the percentage of lymphocyte subsets and the complete blood count and differential. Data are expressed as means ± standard deviation (n=10). No significant differences existed in the pattern of change due to treatment as determined via repeated measures analysis and lsmeans. Within each group, significant differences from pre-exercise ($p<0.05$; indicated by *) were determined via repeated measures analysis and paired t-tests.

Lymphocyte Subset		**Treatment**	**Post-Exercise** (% of total gated cells)	**Effect of Treatment**
CD4	**% CD45RO**	Ginseng	15 ± 9	p = 0.891
		Placebo	14 ± 18	
	% CD45RA	Ginseng	10 ± 9	p = 0.200
		Placebo	21 ± 9	
	RO/RA Ratio	Ginseng	4.7 ± 5.6	p = 0.261
		Placebo	1.4 ± 3.3	
CD8	**% CD45RO**	Ginseng	11 ± 6 *	p = 0.029
		Placebo	4 ± 5	
	% CD45RA	Ginseng	27 ± 17	p = 0.393
		Placebo	22 ± 16	
	RO/RA Ratio	Ginseng	0.5 ± 0.3	p = 0.877
		Placebo	0.4 ± 0.5	

Table 12: Effects of exercise and ginseng on the relative percents of leukocyte common antigen (LCA) isotypes of CD4 and CD8 cells. Lymphocyte subsets from whole blood were determined using prelabelled monoclonal antibodies for specific cell surface markers and flow cytometry. CD45RO is the LCA antigen for memory on T-cells; CD45RA is the LCA antigen for naivety on T-cells. Data are expressed as relative percent means ± standard deviation (n=8). Significant differences between treatments are indicated by (*).

Proliferation Measurement	Treatment	Pre-Exercise	Post-Exercise	Recovery	Effect of Time p-value	Effect of Time x Treatment p-value
48 hr US (dpm)	Ginseng	1197 ± 1001	2975 ± 2419	2809 ± 2191 *	p = 0.0133	p = 0.0644
	Placebo	2336 ± 2263	1964 ± 1168	3840 ± 3090		
48 hr PHA SI	Ginseng	121 ± 161	55 ± 72	49 ± 49	p = 0.0994	p = 0.2524
	Placebo	33 ± 25	36 ± 30	25 ± 15		
72 hr US (dpm)	Ginseng	3316 ± 3926	5142 ± 4362	5622 ± 5016 *	p = 0.0231	p = 0.4496
	Placebo	3995 ±2349	4026 ± 1687	5928 ± 2198		
72 hr PHA SI	Ginseng	94 ± 84	53 ± 41	53 ± 43	p = 0.0261	p = 0.7594
	Placebo	55 ± 41	52 ± 42	35 ± 22		

Table 13. Effects of exercise and treatment on the rate of [^{3}H]-thymidine incorporation by lymphocytes in culture with and without stimulation. Proliferation of lymphocytes was estimated by [^{3}H]-thymidine incorporation (unstimulated; US) and 48 or 72 hours after stimulation with PHA (n=9). The stimulation index (SI) was calculated for both 48 and 72-hour periods. Data are expressed as means ± standard deviation. No significant effects of treatment were present after either 48 or 72-hours, as determined by repeated measures ANOVA. Significant differences ($p < 0.05$) from pre-exercise were detected within groups, indicated by (*).

Treatment	Pre-Exercise (ρg/ml)	Post-Exercise (ρg/ml)	Recovery (ρg/ml)	Effect of Time p-value	Effect of Treatment x Time p-value
Ginseng	200.19 ± 165.94	305.20 ± 170.80	282.17 ± 158.70	p = 0.1101	p = 0.0118
Placebo	230.09 ± 161.96	194.38 ± 140.46	235.34 ± 195.27		

Table 14. Effects of exercise and treatment on *in vitro* lymphocyte interleukin-2 (IL-2) production. Lymphocytes were cultured for 48 hours, either unstimulated or stimulated with PHA and IL-2 production was determined via ELISA (n=8). No IL-2 production was detected for unstimulated lymphocytes (data not shown). Exercise had no significant impact on *in vitro* IL-2 production of lymphocytes after PHA stimulation, as determined by repeated measures ANOVA. Treatment significantly altered the pattern of *in vitro* cytokine production (p=0.0118). No significant differences between treatments at each time point were found, as determined by least square means.

Time after PMA	**Treatment**	**Pre-Exercise** (mean fluorescence)	**Post-Exercise** (mean fluorescence)	**Recovery** (mean fluorescence)	**Effect of Time p-value**	**Effect of Time x Treatment p-value**
0 minutes	Ginseng	16.31 ± 11.55	12.73 ± 2.61	18.78 ± 10.30	p = 0.1099	p = 0.9619
	Placebo	19.62 ± 24.63	13.94 ± 4.41	19.13 ± 14.98		
5 minutes index	Ginseng	23.41 ± 17.89	20.99 ± 15.78	16.64 ± 11.02	p = 0.2783	p = 0.5470
	Placebo	14.56 ± 11.88	14.04 ± 5.21	13.07 ± 10.82		
10 minutes index	Ginseng	68.39 ± 44.61	65.24 ± 44.09	48.43 ± 26.70	p = 0.1845	p = 0.3877
	Placebo	62.07 ± 50.33	47.33 ± 19.94	53.36 ± 53.15		
15 minutes index	Ginseng	101.53 ± 70.80	111.09 ± 40.56	78.92 ± 38.99	p = 0.1718	p = 0.3323
	Placebo	139.33 ± 89.49	99.06 ± 39.98	100.53 ± 100.53		

Table 15. Effect of exercise and treatment on neutrophil oxidative burst activity before and after PMA stimulation. Oxidative burst activity was determined by mean fluorescence of gated cells using flow cytometry before (0 minutes) and after (5, 10, and 15 minutes) PMA stimulation *in vitro* (n=10). Data are expressed as means ± standard deviation. No significant differences existed due to exercise or treatment, as determined using repeated measures ANOVA.

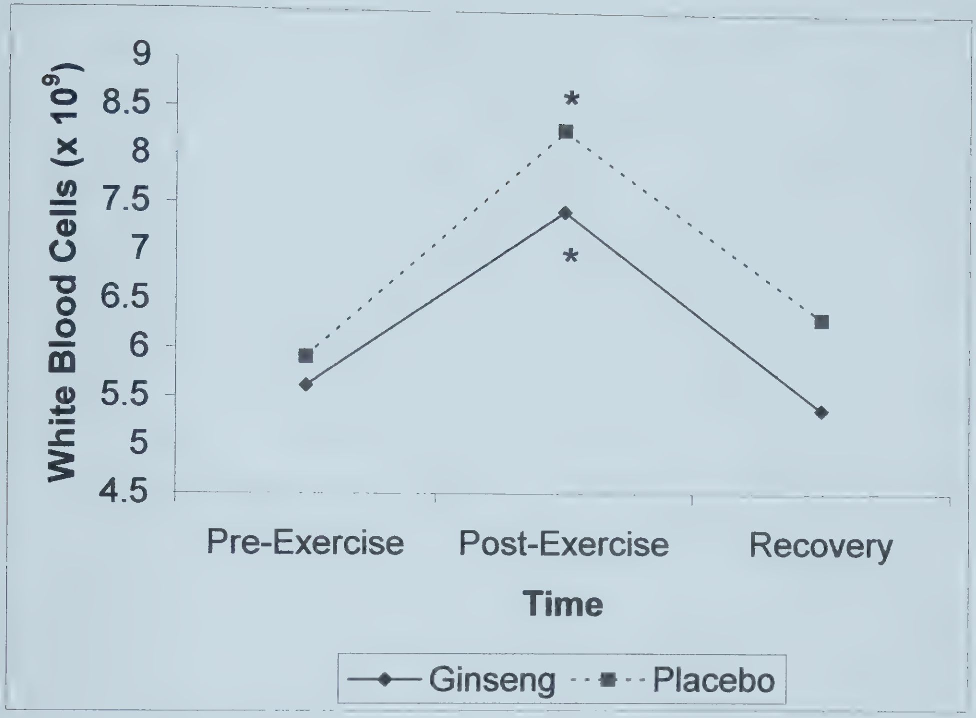

Figure 1: Effects of treatment and exercise on circulating white blood cell (WBC) counts. Data are expressed as means. Within each treatment group, significant differences ($p < 0.05$) from pre-exercise are indicated by (*). No significant differences between ginseng and placebo treatment groups were identified.

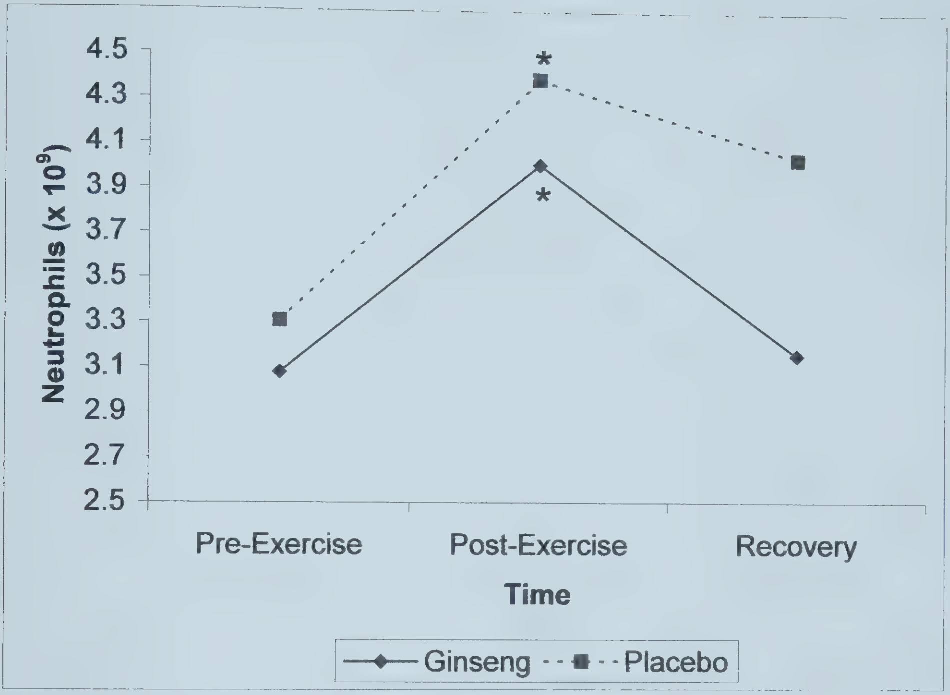

Figure 2: Effects of treatment and exercise on circulating neutrophil counts. Data are expressed as means. Within each treatment group, significant differences ($p < 0.05$) from pre-exercise are indicated by (*). No significant differences between ginseng and placebo treatment groups were identified.

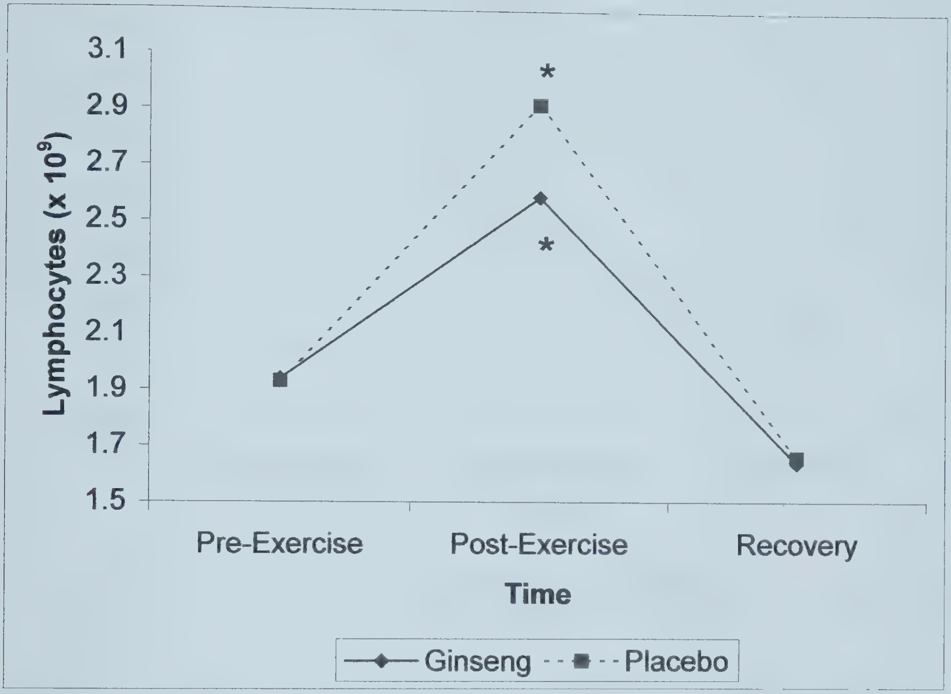

Figure 3: Effects of treatment and exercise on circulating lymphocyte counts. Data are expressed as means. Within each treatment group, significant differences ($p < 0.05$) from pre-exercise are indicated by (*). No significant differences between ginseng and placebo treatment groups were identified.

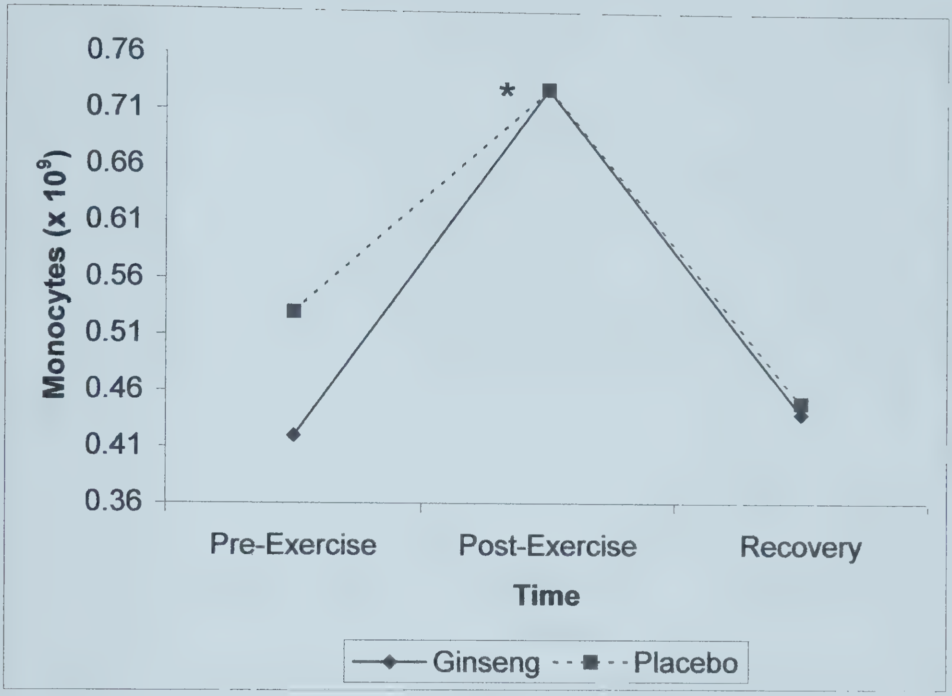

Figure 4: Effects of treatment and exercise on circulating monocyte counts. Data are expressed as means. Within each treatment group, significant differences ($p < 0.05$) from pre-exercise are indicated by (*). No significant differences between ginseng and placebo treatment groups were identified.

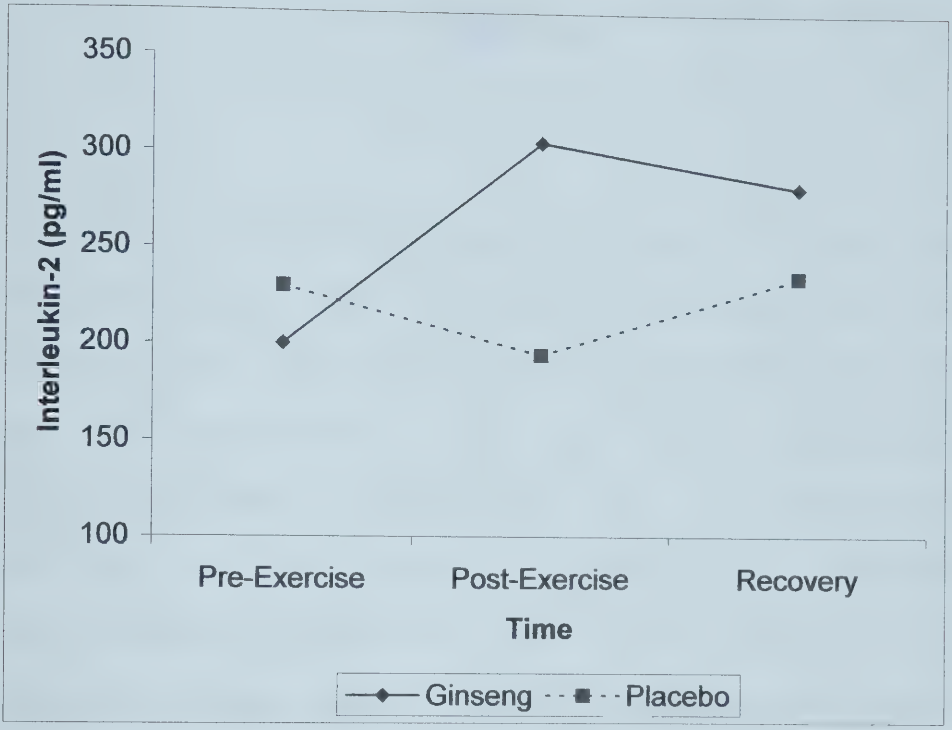

Figure 5: Effects of treatment and exercise on *in vitro* lymphocyte interleukin-2 (IL-2) production. Data are expressed as means. There were no significant differences in IL-2 production due to exercise. Ginseng treatment significantly increased IL-2 production, as determined by repeated measures ANOVA ($p = 0.0118$).

Chapter 5

Discussion

A. Introduction

Ginseng is one of the most popular herbal products in the world. It has been estimated that at least 6 million Americans use ginseng to treat a variety of ailments (O'Hara et al., 1998). Ginseng has been used in Chinese pharmacology for over 2000 years to cure digestive problems, nervous disorders, increase resistance to stress and promote vitality (Bahrke and Morgan, 1994; O'Hara et al., 1998; Goldstein, 1975). Some recent studies have indicated that ginseng has both antineoplastic and immunomodulatory effects (Attele et al., 1999). The vast majority of studies regarding ginseng have used *Panax ginseng* C.A. meyer; few studies exist on the effects of North American ginseng, *Panax quinquefolius*. The immune response to exercise provides an experimental model for determining the effects of physical stress on the immunological responses of humans (Hoffman-Goetz and Pedersen, 1994; Brenner et al., 1999; Mackinnon, 1999). The purpose of the present study was to determine the effects of daily consumption of North American ginseng, *Panax quinquefolius*, on the immune response to acute physiological stress using a moderate exercise protocol.

B. Study Participants

Fourteen males met the inclusion criteria and were enrolled in the present study; ten participants were able to complete both treatment and data collection periods. VO_{2max} and VT, measures of aerobic capacity, were similar between both treatment periods

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

(Table 2). These measures are relevant to the interpretation of the immunological data in that changes in the immune system due to exercise vary with the fitness levels of study participants (Nieman, 1997). Physical activity indices, assessed using the Modified Baecke Activity Questionnaire (Pols et al., 1995), were also similar between treatment periods (Table 4). It has been proposed that exercise training alters the immune response to both an acute bout of exercise and to infection (Nieman, 1997). It is therefore important that study participants maintained similar activity levels during both phases of the present study. The absence of changes in measures of body composition (Table 5) further serve to show that there were no differences within participants over the course of the two treatment arms. Dietary intake of macronutrients has also been shown to modulate the immune response to exercise (Bishop et al., 1999). The macronutrient intakes of study participants also remained the same throughout the study (Table 3).

C. Side Effects of Treatment

A variety of adverse effects have been reported with ginseng administration. These include acute hypertension, behaviour stimulation, sleeplessness, diarrhea, and even atopy (Siegel, 1979). The term "ginseng abuse syndrome" was first used by Siegel (1979) for patients who experienced a variety of adverse effects from the daily consumption of an average of 3 grams of root material. However, few adverse reactions to ginseng are reported and the American Food and Drug Administration has placed ginseng in the "generally recognized as safe" category (O'Hara et al., 1998). It is difficult to discern from the literature whether adverse effects of ginseng are rare or whether they are simply not reported (Engels, 1999). In the present study few adverse

effects of ginseng administration were reported by participants. Two participants reported mild insomnia and hot flashes, which subsided within a week of the onset of ginseng administration. No other side effects were reported by study participants.

D. Effects of Ginseng and Exercise on Immune Parameters

Physical exercise has been shown to promote acute transient alterations in the numbers of peripheral blood leukocytes that are intensity- and duration-dependent (Moyna et al., 1996). According to Gabriel and Kindermann (1997), the leukocytosis of exercise lasting less than two hours can be divided into two phases: during the first phase, the number of leukocytes begins to rise and reaches maximal values within about 30 minutes; during the second phase, or upon the cessation of exercise, there is a rapid decline in the number of lymphocytes and a continued increase in the number of circulating neutrophils. Short duration aerobic exercise induces lesser increases in neutrophil and lymphocyte counts than longer lasting exercise of the same intensity. Similarly, moderate exercise causes a lesser leukocytosis than intense exercise of the same duration (Gabriel and Kindermann, 1997). In general, neutrophil concentrations have been shown to increase during and after exercise, the total lymphocyte count during exercise increases due to recruitment of NK, B, and T-cells, and there is a simultaneous decline in the CD4/CD8 ratio (Hoffman-Goetz and Pederson, 1994). In a study of 21 healthy young men with a mean VO_{2max} of 48 ml/kg/min, Shinkai et al (1996) found that granulocytes, lymphocytes, and monocytes were increased within 30 minutes of moderate exercise (60 minutes at 60% of VO_{2max}). After the cessation of exercise, granulocytes

continued to increase in number in the peripheral circulation, whereas lymphocyte numbers began to decline and even dipped below baseline values.

In the present study, participants exercised for 15 minutes at 80% VT and 15 minutes at 100% VT, which corresponded to a mean of just under 70% VO_{2max}. This moderate exercise protocol induced significant changes in the total number of circulating white blood cells at the end of the exercise period for both ginseng and placebo treatments (post-exercise values; Tables 6). During the recovery period, the number of leukocytes was similar to pre-exercise values for both groups. These changes correspond to the well-documented leukocytosis of exercise (Shinkai et al., 1996; Smith, 1997; Hoffman-Goetz and Pedersen, 1994). The increase in the number of circulating leukocytes post-exercise can be attributed to an increase in the number of neutrophils, lymphocytes, and monocytes in the ginseng treatment. However, in the placebo group significant increases in only neutrophils and lymphocytes could be detected (Tables 7, 8, 9). There were no effects of ginseng treatment on the number or proportion of circulating neutrophils, lymphocytes, or monocytes during the recovery period compared to placebo treatment (Tables 7, 8, 9). Srisurapanon et al. (1997) found that the neutrophil count and total WBC counts were significantly reduced following eight weeks of 300 mg/day *Panax ginseng* extract consumption. However, Scaglione et al. (1996) found that there were no effects of the ingestion of 200 mg of the proprietary G115 *Panax ginseng* extract on the number of leukocytes, neutrophils, or lymphocytes of a large group of study participants. The increases in the total number of white blood cells, neutrophils, and lymphocytes immediately after exercise may represent an increased opportunity for the

immune system to defend against bacteria, viruses, and malignancy. These observations lend support to the concept that moderate exercise reduces the risk of upper respiratory tract infection and some types of malignancies (Pedersen and Toft, 2000).

The monocyte/macrophage system is responsible for the phagocytosis of antigens, the processing and presentation of antigenic fragments to T-cells, and the secretion of cytokines (Gabriel and Kindermann, 1997). Both regular and mature monocytes have been shown to increase in response to various exercise protocols; regular monocytes appear to remain elevated above pre-exercise levels while mature monocytes show a slight decline from pre-exercise levels during the recovery period (Gabriel and Kindermann, 1997). In the present study the number of monocytes, as assessed by complete blood count and differential, increased post-exercise compared to pre-exercise in the ginseng-treated group only. No differences between ginseng and placebo treatment on the proportion of circulating monocytes were found in the present study. Few effects of ginseng administration on monocyte numbers or function have been documented. It is possible that the reported immunostimulatory effects of ginseng are due an increase in the number of circulating monocytes. An increased number of monocytes during exercise, followed by their decline in the recovery period may represent an opportunity for monocytes to enter tissues damaged by exercise (Mackinnon, 1999).

Previous reports (Smith, 1997) have indicated that single episodes of exercise have intensity-dependent effects on neutrophil function. Moderate exercise has been shown to prime the phagocytic and killing capacity of neutrophils, whereas intense

exercise has been shown to reduce some aspects of neutrophil function (Smith, 1997). It has been proposed that moderate exercise triggers different changes in different neutrophil subpopulations (Smith, 1997). In the present study, it was found that there were no significant effects of exercise on neutrophil oxidative burst capacity. It was hypothesized that ginseng treatment would increase neutrophil oxidative burst activity. Scaglione et al. (1990) demonstrated that 8 weeks of daily ingestion of 200 mg *Panax ginseng* significantly increased neutrophil chemotactic activity, phagocytosis, and intracellular microbial killing; it is unclear whether this increase in functional activity was due to an increase in neutrophil cell numbers or due to an increase in the functional activity on a per cell basis. In the present study there was no effect of ginseng administration on neutrophil oxidative burst activity compared to placebo treatment. It is possible that the exercise protocol used was of too moderate intensity to alter neutrophil oxidative burst activity and therefore to detect alterations due to ginseng administration. Future studies should be conducted to confirm the effects of North American ginseng on neutrophil function. In our study, several individuals demonstrated what appeared to be different neutrophil populations based on cell size and granularity. Whether there are neutrophil subpopulations that respond differentially to either ginseng or exercise should be the focus of future research.

Lymphocytes have been shown to be highly responsive to various durations and intensities of exercise. In a study of untrained men with a mean VO_{2max} of 48.0 ml/kg/min, a moderate exercise protocol (60 minutes of cycling at 60% VO_{2max}) resulted in increased NK, T-cytotoxic, T-helper, and B-cell numbers during exercise, with a

subsequent post-exercise decline below baseline values (Shinkai et al., 1996). The CD4/CD8 ratio declined during exercise and returned to baseline values after 2 hours of recovery. The increase in B-cells during exercise was moderate and returned to pre-exercise values soon after the cessation of exercise. The lymphopenia found during the recovery period of the study was due mainly to decreases in CD3+ and CD4+ cell populations. Moyna et al. (1996) examined the effects of a continuous sub-maximal exercise test on the proportion and function of circulating lymphocytes in sedentary and active males and females. They found no differences between sexes or activity levels on either the resting or exercise-induced changes in lymphocytes. These authors found that 18 minutes of sub-maximal exercise resulted in an increase in NK cells and a decline in the number of T-cells, particularly T-helper cells. Their exercise protocol had no impact on the number of CD8+ cells; the decline in the CD4/CD8 ratio was due primarily to the decline in CD4+ cells. The protocol also resulted in a slight decline in the number of B-cells. Normally, it is appropriate for the CD4/CD8 ratio to be 1.5 or greater (Shepard and Shek, 1999). As described above, the CD4/CD8 ratio has been shown to decrease during and after a variety of exercise protocols.

In the present study, there were no significant effects of treatment on the number or relative percent of lymphocyte subtypes. *In vivo* administration of wild *Panax ginseng* was found to increase the percentage of T-helper and T-cytotoxic cells compared to controls (Mizuno et al., 1994). In the present study, lymphocyte subtypes varied in both number and proportion with exercise; the variation within the ginseng group compared to within the placebo group was different. The number of CD3+ T-cells

was increased post-exercise in the placebo group, whereas the proportion of total cells decreased. This change with exercise was not present in the ginseng-treated group. Within the placebo group, the increase in the number of CD3+ cells can be partly accounted for by an increase in the number (but not proportion) of CD8+ cells. In the placebo group, the post-exercise period there were no significant increases in the number of CD4+ cells, however the relative percent of CD4+ lymphocytes had significantly declined. There was therefore a trend towards a decline in the CD4/CD8 ratio post-exercise in the placebo group ($p = 0.07$; Table 10). This decline in the CD4/CD8 ratio following exercise has been well-documented (Mackinnon, 1999; Gabriel and Kindermann, 1997). Within the ginseng group there was no change in either the number or proportion of CD4+ cells or CD8+ cells.

The measurement of CD45RA and CD45RO provides an estimate of the maturation of T-cells (Gabriel and Kindermann, 1997). Both CD4+/CD45RO- and CD4+/CD45RO+ T-cells have been shown to increase during many types of exercise and decline slightly in the recovery period (Gabriel and Kindermann, 1997). The same pattern is true for CD8+/CD45RO- and CD8+/CD45RO+ T-cells, although the effects on T-cytotoxic cells appear to be more dramatic than for T-helper cells (Gabriel and Kindermann, 1997). Liu et al. (1995) found that the relative proportion of CD45RA+ lymphocytes was increased by administration of *Panax ginseng* to healthy young people. They also found that the *in vitro* culture of lymphocytes with *Panax ginseng* increased the relative proportion of both CD45RA+ and CD45RO+ lymphocytes (Liu et al., 1995). In the present study, we examined the expression of these activation markers at only one time point. It was found that ginseng significantly increased the expression of

CD8+/CD45RO+ cells compared to placebo post-exercise. It thus appears that ginseng increases the number of T-cytotoxic cells displaying antigenic memory, although it does not alter the absolute number of T-cytotoxic cells. These cells are more able to respond to peptide-MHC complexes displayed on target cells or antigen presenting cells without stringent requirements for other co-stimulatory signals (Kuby, 1999). It is therefore possible that ginseng increases the ability of the adaptive immune system to respond quickly to foreign antigen or sites of inflammation.

Many studies have examined the effects of exercise on *in vitro* lymphocyte proliferation. *In vivo*, lymphocyte proliferation, stimulated by IL-2 production from T-helper cells, is important for defence against both viral infections and neoplastic cells (Shepard and Shek, 1999). Very intense exercise has been shown to reduce lymphocyte proliferation (Shepard and Shek, 1999). Other authors have noted that the ability of lymphocytes to proliferate *in vitro* following exercise is variable depending on the mitogen used: proliferation has been shown to decrease after exercise in response to concanavalin A (Con A) and to increase in response to IL-2 or lipopolysaccharide (Hoffman-Goetz and Pedersen, 1994). Moyna et al. (1996) found that *in vitro* lymphocyte proliferation following moderate exercise was increased, unchanged, or decreased in response to 1.25 μg/ml, 2.5 μg/ml, and 5.0 μg/ml of Con A respectively. The authors proposed that distinct lymphocyte subsets were activated by different concentrations of Con A, a nonspecific T-cell mitogen. Tvede et al. (1993) found that lymphocytes collected after a moderate cycling exercise protocol had an increased proliferative response to Con A and a decreased proliferative response to PHA compared

to lymphocytes collected at rest. Oshida et al. (1988) also found a decreased proliferative response of lymphocytes to PHA following two hours of cycling at 60% VO_{2max}. In the present study no effects of exercise on the ability of lymphocytes to proliferate *in vitro* were detected in the post-exercise period. During the recovery period, unstimulated lymphocytes demonstrated an increased ability to proliferate after both 48 and 72 hours of culture in the ginseng group only. This may in part be explained by the observation that there was no decline in the number of CD4+ cells in the ginseng group. The increased ability of lymphocytes to proliferate *in vitro* may represent an increased ability to respond to an antigenic challenge. It thus appears that ginseng may improve the ability of lymphocytes to proliferate during recovery from a moderate physical stress by preventing exercise-induced alterations in T-cell subsets.

Scaglione et al. (1990) found that the daily ingestion of 200 mg of *Panax ginseng* for 8 weeks significantly increased the ability of lymphocytes to proliferate *in vitro* in response to Con A, PHA, and LPS. Ginseng itself has been shown to act as a mitogen in *in vitro* lymphocyte proliferation studies. Mizuno et al. (1994) reported that culture of lymphocytes with wild *Panax ginseng* increased their ability to proliferate in a dose response manner. Liu et al. (1995) demonstrated that incubation with low doses of Rg1 isolated from *Panax ginseng* resulted in an increased ability of lymphocytes to proliferate *in vitro* in response to PHA. American ginseng has been shown to increase lymphocyte proliferation *in vitro* in concentrations ranging from 10^{-2} to 10^{-6}, whereas culture with *Panax ginseng* had no effect on the proliferative response of lymphocytes (Borchers et al., 1998). The same study found that neither American ginseng nor *Panax*

ginseng had cytotoxic effects on lymphocytes at any dose tested. It has been postulated that differing concentrations of particular ginsenosides are responsible for the alterations of *in vitro* lymphocyte proliferation (Borchers et al., 1998). It appears that in the present study lymphocytes are stimulated *in vivo* by ginseng, given that at recovery from exercise unstimulated lymphocyte proliferation increased (Table 13) after both 48 and 72 hours. It is unclear at present what concentrations of ginsenosides appear in the circulation or how these compounds interact with lymphocytes to produce alterations in lymphocyte proliferation following ingestion.

The effects of exercise on IL-2 production both *in vitro* and *in vivo* have been studied. IL-2 is important for the stimulation of NK, LAK, and T-cell function (Shepard and Shek, 1999). In general, *in vitro* studies have shown that IL-2 is decreased following exercise and that this may be due to an increase in the number of lymphocytes expressing the IL-2 receptor (Shepard and Shek, 1999). Tvede et al. (1993) demonstrated that moderate exercise at 25%, 50%, and 75% of VO2max resulted in a decrease, no change, and an increase in the IL-2 production of lymphocytes compared to resting values. Similarly, Shepard et al. (1994) reported that the effects of exercise on IL-2 production appear to be intensity-dependent. In the present study, the pattern of *in vitro* IL-2 production was significantly altered by the interaction of treatment and exercise. In the ginseng-treated group there was a tendency for IL-2 production to increase post-exercise whereas IL-2 production tended to decrease post-exercise in the placebo group (Table 14). It is possible that this difference is due to a decrease in the relative proportion of CD4+ lymphocytes in the placebo group post-exercise (Table 10). The increased ability

of lymphocytes to produce IL-2 immediately following a moderate physical stress may reflect an increased ability of lymphocytes to respond to an immunological challenge in the ginseng group. Other studies examining the effects of ginseng administration on IL-2 production were not found, however culture of lymphocytes with *Panax ginseng* has been shown to increase the expression of IL-2 receptors (Liu et al., 1995). *In vivo, Panax ginseng* administration was found to have no effect on the expression of the IL-2 receptor (Srisurapanon et al., 1997). Culture of macrophages in the presence of *Panax ginseng* has been shown to increase the production of IL-1 in a manner similar to LPS (Kenarova et al., 1990). The *in vitro* production of IL-1 was not determined in the present study, but may be of interest in the future. The effects of exercise on IL-6, TNF-α, and IL-10 have been well studied (for example, Brenner et al., 1999). It is recommended that these cytokines be evaluated in future studies, in order to further elucidate the manner in which ginseng influences the Th1 and the Th2 response pathways.

B-cells are primarily responsible for antibody production (Gabriel and Kindermann, 1997). The response of B-cells to exercise has generally been less well studied than that of T-cells. The response of B-cells to exercise is less pronounced than that of other lymphocyte subpopulations (Mackinnon, 1999). In general, B-lymphocyte numbers appear to increase slightly during brief periods (< 45 minutes) of exercise and decline slightly in the recovery period (Mackinnon, 1999; Gabriel and Kindermann, 1997). In the present study, the proportion of B-cells was significantly reduced post-exercise within each of the ginseng and placebo-treated groups. The placebo-treated

group demonstrated a significant increase in the proportion of B-cells in the recovery period compared to the pre-exercise period; this difference was not detected in the ginseng-treated group. The number of B-cells was increased post-exercise in the placebo group. The changes in both the number and proportions of B-cells induced by exercise were small compared to the changes in other lymphocyte subsets, as has been previously demonstrated (Rowbottom and Green, 2000). There were no effects of ginseng treatment, as determined by repeated measures analysis, on the number of circulating B-cells. Similarly, Srisurapanon et al. (1997) found no effects of the daily ingestion of 300 mg *Panax ginseng* for eight weeks on the number of circulating B-cells. However, Scaglione et al. (1996) found a significantly increased antibody titer, a measure of B-cell function, in response to influenza vaccination in subjects receiving 200 mg of *Panax ginseng* for 12 weeks compared to those taking placebo. Antibody titers have also been found to be increased in mice treated with *Panax ginseng* (Singh et al., 1984 and Kenarova et al., 1990). In future studies, it would be of value to examine the effects of ginseng administration on B cell function following exercise.

Natural killer cell concentrations and proportions have been shown to be highly responsive to exercise (Rowbottom and Green, 2000; Mackinnon, 1999). The effects of exercise on NK cells are exercise intensity dependent (Shepard and Shek, 1999). Immediately following moderate exercise, both the concentration of NK cells and the natural killer cell activity have been shown to increase (Gabriel and Kindermann, 1997). During recovery from exercise, the NK cell count and cytolytic activity decline; these values return to pre-exercise levels anywhere from one to 24 hours after exercise,

depending on the duration of the activity (Shepard and Shek, 1999). Scaglione et al. (1990) demonstrated that administration *Panax ginseng* extracts for 8 weeks resulted in increased natural killer cell activity, as determined by % ^{51}Cr released by target cells. In the present study the number of CD16+ lymphocytes increased dramatically post-exercise in both the ginseng and placebo treatment groups, as we would expect based on previous studies. However, the proportion of CD16+ cells was increased in only the ginseng group post-exercise. Ginseng ingestion may therefore increase the proportion of circulating NK cells during moderate exercise and represent an increased opportunity for defence against micro-organisms and certain tumour cells. Future studies should examine the effects of ginseng administration on both natural killer cell concentration and cytotoxicity.

E. General Discussion

The present study employed a double-blind, placebo-controlled cross-over design in order to determine the effects of ginseng administration on immune function. We encountered several difficulties with the use of this study design. First, the time commitment required of study participants was approximately six months long and included a three-month washout-period. Study participants were required to take capsules for 35 days and were actively involved with the researchers for only 3 – 4 days of each treatment arm. It is possible that the lack of active participation in the study resulted in the attrition of several participants. Second, it was difficult to verify that study participants consumed the capsules according to study directions. Compliance with regard to capsule intake was monitored by counting unused capsules at the end of each

study period and appeared to be very high. It is not known whether the capsules were consumed 3 times daily with meals, as requested. Furthermore, one participant admitted to breaking open the capsule at the end of a treatment period “just to see what was in it”. It is suggested that future studies provide weekly supplies of capsules. This would allow researchers to meet with participants on a more frequent basis to ensure compliance with instructions and to maintain participants’ interest in the study.

There is considerable variation in the literature with respect to ginseng harvesting, manufacturing, and dosage. It has been shown that cultivation and harvesting, as well as manufacturing practices have an impact on the ginsenoside content of ginseng preparations (Liu and Xiao, 1992). Since ginsenosides are the main active constituent of ginseng, it is important that their content be standardized in preparations used for research purposes. In the present study, capsules were standardized in terms of manufacturing and content and were from the same lot. It is important that future studies document not only the species of ginseng, but also the content of the various ginsenosides in order for meaningful comparisons between studies to be made. Furthermore, the dose of ginseng administered in the present study was based upon the manufacturers recommendations and a review of the literature. At present there exists no universal recommendation with regard to ginseng, in particular North American ginseng. It is recommended that future studies devise a method for administering ginseng on a body weight basis in order to ensure that study participants are receiving an optimal dose of the herb.

The present study demonstrated that North American ginseng alters the immune response to acute exercise. Ginseng administration did not alter the total number of circulating leukocytes, neutrophils, or lymphocytes following a moderate exercise protocol. However, ginseng administration increased the number of circulating monocytes following exercise. Ginseng administration prevented exercise-induced declines in the proportion of CD3+ and CD4+ cells and increased the porportion of CD16+ cells after a moderate physical stress. Ginseng treatment also increased the proportion of CD8+/CD45RO+ lymphocytes. Ginseng administration increased the functional capacity of lymphocytes following exercise, as determined by an increased ability of unstimulated lymphocytes to proliferate *in vitro* and an increased ability of PHA-stimulated lymphocytes to produce IL-2 *in vitro*. It is therefore possible that ginseng administration increases the ability of the immune system to defend against foreign antigen or certain tumour cells in response to a physical stress.

References

Atopkina LN, Malinovskaya GV, Elyakov GB, Uvarova NI, Woerdenbag HJ, Koulman A, Potier P. Cytotoxicity of natural ginseng glycosides and semisynthetic analogues. Planta Medica 1999; 65: 30 – 34.

Attele AS, Wu JA, Yuan C-S. Ginseng pharmacology. Biochemical Pharmacology 1999; 58: 1685 – 1693.

Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. American Journal of Clinical Nutrition 1982; 36 (5): 936 – 942.

Baranov AI. Medicinal uses of ginseng and related plants in the Soviet Union: recent trends in the Soviet literature. Journal of Ethnopharmacology 1982; 6: 339 – 353.

Baum M, Geitner T, Liesen H. The role of the spleen in the leukocytosis of exercise: consequences for physiology and pathophysiology. International Journal of Sports Medicine 1996; 17 (8): 604 – 607.

Bishop NC, Blannin AK, Walsh NP, Robson PJ, Gleeson M. Nutritional aspects of immunosuppression in athletes. Sports Medicine 1999; 28 (3): 151 – 176.

Bhambhani Y and Singh M. Ventilation threshold during a graded exercise test. Respiration 1985; 47: 120 – 128.

Bharke MS and Morgan WP. Evaluation of the ergogenic properties of ginseng. Sports Medicine 1994; 18 (4): 229 – 248.

Borchers At, Van de Water J, Kenny TP, Keen CL, Stern JS, Hackman RM, Gershwin ME. Comparative effects of three species of ginseng on human peripheral blood lymphocyte proliferative responses. International Journal of Immunotherapy 1998; 14 (3): 143 – 152.

Brekhman II and Dardymov IV. Pharmacological investigation of glycosides from ginseng and eleutherococcus. Lloydia 1969; 32 (1): 46 – 51.

Brenner IKM, Natale VM, Vasiliou P, Moldoveanu AI, Shek PN, Shepard RJ. Impact of three different types of exercise on components of the inflammatory response. European Journal of Applied Physiology 1999; 80: 452 – 460.

Brozek J, Grande F, Anderson JT, Keys A. Densitometric analysis of body composition: revision of some quantitative assumptions. Annals of the New York Academy of Science 1963; 110: 113 – 140.

Bruneton J. Pharmacognosy Phytochemistry Medicinal Plants. Paris, France: Lavoisier; 1995.

Chang H-M, But PP-H. Pharmacology and Applications of Chinese Materia Medica. Singapore, Republic of China: World Scientific Press; 1986.

Dubick MA. Historical perspectives on the use of herbal preparations to promote health. Journal of Nutrition 1986; 116 (7): 1348 – 1354.

Durden Beltz S, Doering PL. Efficacy of nutritional supplements used by athletes. Clinical Pharmacy 1993; 12 (12): 900 – 908.

Engels H-J. Publication of adverse events in exercise studies involving nutritional agents. International Journal of Sport Nutrition 1999; 9: 89 – 91.

Fan Z-H, Isobe K-I, Kiuchi K, Nakashima I. Enhancement of nitric oxide production from activated macrophages by a purified form of ginsenoside (Rg1). American Journal of Chinese Medicine 1995; 23(3-4): 279 – 287.

Gabriel H and Kindermann W. The acute immune response to exercise: what does it mean. International Journal of Sports Medicine 1997; 18 (Suppl. 1): S28-S45.

Goldstein B. Ginseng: its history, dispersion, and folk tradition. American Journal of Chinese Medicine 1975; 3 (3): 223 – 234.

Hoffman-Goetz L and Pederson BK. Exercise and the immune system: a model of the stress response. Immunology Today 1994; 15: 382 – 387.

Hou JP. The chemical constituents of ginseng plants. Comparative Medicine East and West 1977; 5 (2): 123 – 145.

Humphreys JD. North American ginseng and the stress response during acute exercise. 2000.

Kenarova B, Neychev H, Hadjivanova C, Petkov VD. Immunomodulating activity of ginsenoside Rg1 from Panax ginseng. Japanese Journal of Pharmacology 1990; 54: 447 – 454.

Kitts DD, Wijewickreme AN, Hu C. Antioxidant properties of a North American ginseng extract. Molecular and Cellular Biochemistry 2000; 203: 1 – 10.

Kuby J. Immunology, Third Edition. New York: WH Freeman and Company, 1999. pp 3 – 22, 47 – 81.

Liu J, Wang S, Liu H, Yang L, Nan G. Stimulatory effect of saponin from *Panax ginseng* on immune function of lymphocytes in the elderly. Mechanisms of Ageing and Development 1995; 83: 45 – 53.

Liu C-X, Xiao P-G. Recent advances on ginseng research in China. Journal of Ethnopharmacology 1992; 36: 27 – 38.

Mackinnon LT. Advances in Exercise Immunology. Champaign, IL: Human Kinetics, 1999.

Miles MP, Naukam RJ, Hackney AC, Clarkson PM. Blood leukocyte and glutamine fluctuations after eccentric exercise. International Journal of Sports Medicine 1999; 20: 322 – 327.

Mills PJ, Rehman J, Ziegler MG, Carter SM, Dimsdale JE, Maisel AS. Nonselective β-blockade attenuates the recruitment of CD62L$^-$ T lymphocytes following exercise. European Journal of Applied Physiology 1999; 79: 531 – 534.

Mizuno M, Yamada J, Terai H, Kozukue N, Lee YS, Tsuchida H. Differences in immunomodulating effects between wild and cultured Panax ginseng. Biochemical and Biophysical Research Communications 1994; 200 (3): 1672 – 1678.

Motley HL. Comparison of a simple helium closed circuit with the oxygen open circuit method for measuring residual air. American Review of Tuberculous and Pulmonary Diseases 1957; 76: 701 – 715.

Moyna NM, Bodnar JD, Goldberg HR, Shurin MS, Robertson RJ, Rabin BS. Relation between aerobic fitness level and stress induced alterations in neuroendocrine and immune function. International Journal of Sports Medicine 1999; 20: 136 – 141.

Moyna NM, Acker GR, Fulton JR, Weber K, Goss FL, Robertson RJ, Tollerud DJ, Rabin BS. Lymphocyte function and cytokine production during incremental exercise in active and sedentary males and females. International Journal of Sports Medicine 1996; 17 (8): 585 – 591.

Nieman DC. Exercise immunology: practical applications. International Journal of Sports Medicine 1997a; 18 (Suppl. 1): S91 – S100.

Nieman DC. Immune response to heavy exertion. Journal of Applied Physiology 1997b; 82 (5): 1385 – 1394.

Nieman DC, Miller AR, Henson DA, Warren BJ, Gusewitch G, Johnson RL, Davis JM, Butterworth DE, Herring JL, Nehlsen-Cannarella SL. Effect of high- versus moderate-intensity exercise on lymphocyte subpopulations and proliferative response. International Journal of Sports Medicine 1994; 15 (4): 199 – 206.

O'Hara MA, Kiefer D, Farrell K, Kemper K. A review of 12 commonly used medicinal herbs. Archives of Family Medicine 1998; 7 (6): 523 – 536.

Oshida Y, Yamanouchi K, Hayamizu S, Sato Y. Effect of acute physical exercise on lymphocyte subpopulations in trained and untrained subjects. International Journal of Sports Medicine 1988; 9: 137 – 140.

Pedersen BK and Toft AD. Effects of exercise on lymphocytes and cytokines. British Journal of Sports Medicine 2000; 34: 246 – 251.

Pedersen BK, Bruunsgaard H, Klokker M, Kappel M, MacLean DA, Nielson HB, Rohde T, Ullum H, Zacho M. Exercise-induced immunomodulation – possible roles of neuroendocrine and metabolic factors. International Journal of Sports Medicine 1997; 18 (Suppl.1): S2 – S7.

Pols MA, Peeters PHM, Bueno-de-Mesquita HB, Ocke MC, Wentink CA, Kemper HCG, Collette HJA. Validity and repeatability of a modified Baecke questionnaire on physical activity. International Journal of Epidemiology 1995; 24 (2): 381 – 387.

Rowbottom DG and Green KJ. Acute exercise effects on the immune system. Medicine and Science in Sports and Exercise 2000; 32 (7 Suppl.): S396 – S405.

Scaglione F, Cattaneo G, Alessandria M, Cogo R. Efficacy and safety of the standardized ginseng extract G115 for potentiating vaccination against common cold and /or influenza syndrome. Drugs in Experimental Clinical Research 1996; 22 (2): 65 – 72.

Scaglione F, Ferrara F, Dugnani S, Falchi M, Santoro G, Fraschini F. Immunomodulatory effects of two extracts of Panax ginseng C.A. Meyer. Drugs in Experimantal Clinical Research 1990; 16 (10): 537 – 542.

Shepard RJ and Shek PN. Exercise, immunity, and susceptibility to infection. The Physician and Sports Medicine 1999; 27 (6): 47 – 71.

Shepard RJ and Shek PN. Associations between physical activity and susceptibility to cancer. Sports Medicine 1998; 26 (5): 293 – 315.

Shepard RJ, Rhind S, Shek PN. Exercise and training: influences on cytotoxicity, interleukin-1, interleukin-2 and receptor structures. International Journal of Sports Medicine 1994; 15: S154 – S166.

Shinkai S, Watanabe S, Asai H, Shek PN. Cortisol response to exercise and post-exercise expression of blood lymphocyte subset counts. International Journal of Sports Medicine 1996; 17 (8): 597 – 603.

Siegel RK. Ginseng abuse syndrome. Journal of the American Medical Association 1979; 241 (15): 1614 – 1615.

SinghVK, Agarwal SS, Gupta BM. Immunomodulatory activity of Panax ginseng extract. Planta Medica 1984; 48: 462 – 465.

Singh VK, George CX, Singh N, Agarwal SS, Gupta BM. Combined treatment of mice with Panax ginseng extract and interferon inducer. Planta Medica 1983; 47: 234 – 236.

Siri WE. Gross composition of the body. In: Lawrence JH and Tobias CA (eds.) Advances in Biological and Medical Physics, Volume IV. New York: Academic Press 1956.

Smith JA. Exercise immunology and neutrophils. International Journal of Sports Medicine 1997; 18 (Suppl.1): S46 – S55.

Srisurapanon S, Apibal S, Siripol R, Rungroeng K, Cherdrugsi P, Vanich-Angkul V, Timvipark C. The effect of standardized ginseng extract on peripheral blood leukocytes and lymphocyte subsets: a preliminary study in young healthy adults. Journal of the Medical Association of Thailand 1997; 80 (1): S81 – S85.

Thoden JS. Testing aerobic power. In: MacDougall JD, Wenger HA, Green HJ (eds.) Physiological testing of the high-performance athlete, Second Edition. Champaign, IL: Human Kinetics 1991: 108 – 173.

Tvede N, Kappel M, Halkjoer-Kristensen J, Galbo H, Pedersen BK. The effects of light, moderate and sever bicycle exercise on lymphocyte subsets, natural and lymphokine activated killer cells, lymphocyte proliferative response and interleukin-2 production. International Journal of Sports Medicine 1993; 14 (5): 275 – 282.

Vigano C, Ceppi E. What is ginseng. The Lancet 1994; 344: 619.

Wong AHC, Smith M, Boon HS. Herbal remedies in psychiatric practice. Archives of General Psychiatry 1998; 55: 1033 – 1044.

Appendix A
Recruitment Poster

University of Alberta
Faculty of Agriculture, Forestry, and Home Economics
Faculty of Physical Education and Recreation

Ginseng and Exercise Study

Volunteers are needed for a study on ginseng and exercise. If you volunteer and meet the study entry criteria, you will receive:

FREE BODY COMPOSITION ASSESSMENT!

FREE AEROBIC FITNESS TESTING!

FREE DIETARY ASSESSMENT!

Are you:

- In Good Health?
- Male?
- 18-35 Years Old?
- Low to Moderately Active?
- Willing to give Blood Samples?

For More Information, Please Contact:

Sarah Robbins @ 492-4267 (email: sarah@ualberta.ca)
Jen Humphreys @ 492-8739 (email: jdh6@ualberta.ca)

Appendix B
Demographic Questionnaire

DEMOGRAPHIC QUESTIONNAIRE

Name:______________________________ Date:______________________________

Address:____________________________ Telephone (Home):__________________

____________________________________ (Work):__________________

____________________________________ E-mail:____________________________

Date of Birth:_______________________ Age:_______________

The Investigators will need to determine your:

Height:_______(cm) Weight:_______(Kg) BMI:_______

Please answer the following questions as accurately as possible. Please ask for clarification where needed.

1. Have you experienced a weight loss or gain (+/- 3Kg or 6.6lbs or more) in the last 2 months?
   Yes________ No_______
   If yes, specify the amount of weight lost (-) or gained (+) __________________

2. List any prescribed medication or over-the-counter medication you regularly take:
____
____
____
____

3. List any vitamin supplements, herbal supplements, or other dietary supplements that you take (please include herbal teas, cold remedies, weight gain/loss supplements):
____
____
____
____

4. Do you have a heart, liver, or renal disease? Yes_______ No_______

5. Do you have diabetes or a thyroid disorder? Yes_______ No_______

6. Do you have an autoimmune disease? Yes_______ No_______

7. Do you have any chronic or "nagging" musculoskeletal aches or pains (eg. Sore knees, weak back)?

   Yes_______ No_______
   If yes, indicate the location of your ache or pain and describe any related physical limitations:
   ____
   ____

8. Are you allergic to any drugs, food or beverages? Yes______ No______
If yes, please list:____
____

9. Have you ever, or do you currently, take ginseng? Yes______ No______
If yes, please describe the type, amount, and duration that you have taken ginseng:
____
____

10. Do you smoke: Yes______ No______

11. Are you comfortable putting your head under water? Yes______ No______

12. Are you afraid of needles? Yes______ No______

Physical Activity

1. Are you involved in a regular routine of physical activity? Yes______ No______

If "YES"...

(a) Does your routine include **4 or more sessions** a week? Yes_____ No______
For how long have you been doing this routine? ________________

(b) Does your routine **exceed 3 hours** a week? Yes______ No______
For how long have you been doing this routine? ________________

2. Please list and describe ALL of the physical activities that you are involved in:

ACTIVITY	DURATION (MIN/SESSION)	FREQUENCY (SESSIONS/WEEK)	INTENSITY*
Eg. Running	20	4	1 2 3 4 5
1.			1 2 3 4 5
2.			1 2 3 4 5
3.			1 2 3 4 5
4.			1 2 3 4 5
5.			1 2 3 4 5
6.			1 2 3 4 5

*INTENSITY: 1 – Not vigorous at all (very light) 2 – Somewhat vigorous (light)
3 – Moderately vigorous (medium) 4 – Vigorous (heavy)
5 – Extremely vigorous (very heavy)

Subject Availability

All tests and exercise sessions will be scheduled at your convenience. Remember that the test day will take from 9am-5pm. Please indicate the days when you are available for testing and exercise sessions. You will be required to attend a testing day at the end of each ginseng treatment period.

TIME SLOT	MON	TUES	WED	THURS	FRI	SAT	SUN
9am-5pm							

Please feel free to add other comments you think are important for us to know:

____

____

____

____

Appendix C
Study Information and Consent Form

UNIVERSITY OF ALBERTA
FACULTY OF AGRICULTURE, FORESTRY, AND HOME ECONOMICS
FACULTY OF PHYSICAL EDUCATION AND RECREATION

Effects of ginseng on stress response and immune response.

Investigators: Linda McCargar, Catherine Field, Vicki Harber, Sarah Robbins, Jen Humphrys

Study Information Sheet

Purpose

Ginseng-user claim that regular use of the herb will reduce stress, strengthen metabolism, increase physical stamina, and stimulate the immune system. Many of these claims have not been scientifically documented. In addition, there is little information specifically on the North American variety of ginseng, *Panax quinquefolius.*

The purpose of our study is to investigate the effects of daily consumption of an oral dose of *Panax quinquefolius* on stress response and immune response after acute physiological stress (an intense exercise protocol).

Study Protocol

I. ENTRY CRITERIA

- good health (ie: free of diabetes, thyroid, autoimmune disease)
- male
- ages 18-35 years
- ability to perform strenuous activity
- not taking any medications or herbal supplements
- non-smokers
- VO2 max less than 50 ml/kg/min as determined by an aerobic fitness assessment: Maximal aerobic consumption (VO2 max) will be determined through progressive exercise to volitional fatigue on a cycle ergometer. Muscle discomfort/soreness, shortness of breath, and abnormal heart beat and blood pressure are possible side effects associated with maximal aerobic consumption (VO2 max), but are rare in healthy young individuals. (TIME = 1 HOUR)
- Body Mass Index (BMI) of 20-27: BMI will be obtained through the administration of a demographic questionnaire, at which time height and weight will also be taken.

II. GINSENG AND PLACEBO TREATMENT

You will receive ginseng capsules for one month and placebo capsules for one month. Some side effects of the ginseng capsule that may be experienced include: irritability, insomnia, dizziness, hypertension, and diarrhea. If you experience any of these symptoms, please contact one of the investigators immediately.

III. ONE-DAY STUDY PROTOCOL – These tests will be scheduled and conducted in one day for a total time commitment of approximately 8 hours. You will be required to complete these tests at the end of each of the two treatment periods.

Study Timeline:

Subject Recruitment	**Phase 1: One-Month Ginseng or Placebo Treatment**	**Phase 2: Three-Month Wash-out Period**	**Phase 3: One-Month Ginseng or Placebo Treatment**
Demographic Questionnaire	Three-Day Food Records		Three-Day Food Records
VO2 max testing	Baecke Activity Questionnaire		Baecke Activity Questionnaire
	Resting Energy Expenditure Measurement		Resting Energy Expenditure Measurement
	Under Water Weighing		Under Water Weighing
	Exercise Stress Protocol, Blood and Urine Collection		Exercise Stress Protocol, Blood and Urine Collection

1. **Assessment of Dietary Intake:** You will be instructed how to record your food intake for the duration of two week-days and one weekend day. You will return the completed food records to the investigators on the testing day. (TIME = 2 HOURS)

2. **Self-Report Activity Records:** On the day of testing, you will be asked to complete the Modified Baecke Questionnaire on Physical Activity. It is short and easy to complete and provides an index of the amount of activity spent during work, sports, and leisure. (TIME = 30 MINUTES)

3. **Resting Energy Expenditure (REE):** Resting metabolic rate will be taken in the metabolic testing laboratory in the Agriculture-Forestry Building. The measurements will be taken immediately upon your arrival at the University. You will be required to fast and refrain from exercise 12 hours prior to arriving ath the test site by motor vehicle. After resting on a cot in the supine position for 30 minutes, a transparent hood will be placed over your head and the test started. The test, which will measure inhaled oxygen and exhaled carbon dioxide, will last approximately 30 minutes. (TIME = 1 HOUR)

4. **Light Snack:** You will be provided with a standardized light snack, prepared in the Agriculture-Forestry Building (TIME = 30 MINUTES)

5. **Underwater Weighing:** Body density will be measured by underwater weighing at the University of Alberta. A swimsuit is worn and the water is pleasantly warm. Changing facilities are located adjacent to the test pool. You will be briefly submerged underwater approximately 6 times in order to get a constant measure of your body density. (TIME = 1.5 HOURS)

6. **Standardized Meal:** You will be provided with a standardized lunch prepared in the Agriculture-Forestry Building (TIME = 1 HOUR)

7. **Exercise Stress Protocol, Blood, and Urine Collection:** You will perform 30 minutes of continuous exercise on a stationary bicycle. An intravenous catheter will be inserted in your arm prior to the start of the test, so that blood samples can be taken before, during, and after the exercise. The total amount of blood taken will be equivalent to 70 ml. (Blood donations usually take 500ml). The exercise itself will be of a moderate nature, with some physical exertion

Total time commitment per subject will be approximately 8 hours x 2 days. The initial screening day will take approximately 3 hours. The study will last five months; this period will include two one-month treatment phases and a non-treatment phase of three months.

Confidentiality

The confidentiality of all data and subjects' identities will be ensures. All data will be locked in a filing cabinet in a locked office to which only the principle investigators will have access.

We strongly encourage questions for clarity and understanding of the above outlined study.
For further information, please feel free to contact:

[redacted]

UNIVERSITY OF ALBERTA
FACULTY OF AGRICULTURE, FORESTRY, AND HOME ECONOMICS
FACULTY OF PHYSICAL EDUCATION AND RECREATION

Effects of ginseng on stress response and immune response.

Investigators: Linda McCargar, Catherine Field, Vicki Harber, Sarah Robbins, Jen Humphrys

SUBJECT CONSENT FORM

This study has been satisfactorily explained to me and I understand the necessity for the protocol outlined in the Study Information Sheet. I know that I may contact the persons designated on this form at any time if I have any further questions. I have been informed of the possible benefits of joining this research as well as the possible risks and discomforts. I have been assured that the information obtained from my participation in this study may be published in medical reports, but that my personal records will be kept confidential. I understand that I am free to withdraw from this study at any time without prejudice. I understand that I will be promptly informed of any findings which may develop during the research period that may affect my willingness to continue participating in the study. I understand that I will be given a copy of the Study Information Sheet and the signed Consent Form to keep.

Subject Name (print)	Subject Signature	Date
Witness Name (print)	Witness Signature	Date
Investigator Name (print)	Investigator Signature	Date

Appendix D
Physical Activity and Readiness Questionnaire

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 1994)

PAR - Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
☐	☐	2.	Do you feel pain in your chest when you do physical activity?
☐	☐	3.	In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5.	Do you have a bone or joint problem that could be made worse by a change in your physical activity?
☐	☐	6.	Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7.	Do you know of any other reason why you should not do physical activity?

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

Please note: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

You are encouraged to copy the PAR-Q but only if you use the entire form

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction.

NAME ______________________________

SIGNATURE ______________________________

DATE ______________________________

SIGNATURE OF PARENT ______________________________
or GUARDIAN (for participants under the age of majority)

WITNESS ______________________________

continued on other side...

Supported by: Health Canada Santé Canada

...continued from other side

PAR - Q & YOU

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 1994)

We know that being physically active provides benefits for all of us. Not being physically active is recognized by the Heart and Stroke Foundation of Canada as one of the four modifiable primary risk factors for coronary heart disease (along with high blood pressure, high blood cholesterol, and smoking). People are physically active for many reasons — play, work, competition, health, creativity, enjoying the outdoors, being with friends. There are also as many ways of being active as there are reasons. What we choose to do depends on our own abilities and desires. No matter what the reason or type of activity, physical activity can improve our well-being and quality of life. Well-being can also be enhanced by integrating physical activity with enjoyable healthy eating and positive self and body image. Together, all three equal VITALITY. So take a fresh approach to living. Check out the VITALITY tips below!

Active Living:

- accumulate 30 minutes or more of moderate physical activity most days of the week
- take the stairs instead of an elevator
- get off the bus early and walk home
- join friends in a sport activity
- take the dog for a walk with the family
- follow a fitness program

Healthy Eating:

- follow Canada's Food Guide to Healthy Eating
- enjoy a variety of foods
- emphasize cereals, breads, other grain products, vegetables and fruit
- choose lower-fat dairy products, leaner meats and foods prepared with little or no fat
- achieve and maintain a healthy body weight by enjoying regular physical activity and healthy eating
- limit salt, alcohol and caffeine
- don't give up foods you enjoy — aim for moderation and variety

Positive Self and Body Image:

- accept who you are and how you look
- remember, a healthy weight range is one that is realistic for your own body make-up (body fat levels should neither be too high nor too low)
- try a new challenge
- compliment yourself
- reflect positively on your abilities
- laugh a lot

Enjoy eating well, being active and feeling good about yourself. That's VITALITY.

FITNESS AND HEALTH PROFESSIONALS MAY BE INTERESTED IN THE INFORMATION BELOW.

The following companion forms are available for doctors' use by contacting the Canadian Society for Exercise Physiology (address below):

The Physical Activity Readiness Medical Examination (PARmed-X) - to be used by doctors with people who answer YES to one or more questions on the PAR-Q.

The Physical Activity Readiness Medical Examination for Pregnancy (PARmed-X for PREGNANCY) - to be used by doctors with pregnant patients who wish to become more active.

References:

Arraix, G.A., Wigle, D.T., Mao, Y. (1992). Risk Assessment of Physical Activity and Physical Fitness in the Canada Health Survey Follow-Up Study. **J. Clin. Epidemiol.** 45:4 419-428.

Mottola, M., Wolfe, L.A. (1994). Active Living and Pregnancy, In: A. Quinney, L. Gauvin, T. Wall (eds.), **Toward Active Living: Proceedings of the International Conference on Physical Activity, Fitness and Health**. Champaign, IL: Human Kinetics.

PAR-Q Validation Report, British Columbia Ministry of Health, 1978.

Thomas, S., Reading, J., Shephard, R.J. (1992). Revision of the Physical Activity Readiness Questionnaire (PAR-Q). **Can. J. Spt. Sci.** 17:4 338-345.

To order multiple printed copies of the PAR-Q, please contact the

Canadian Society for Exercise Physiology
185 Somerset St. West, Suite 202
Ottawa, Ontario CANADA K2P 0J2
Tel. (613) 234-3755 FAX; (613) 234-3565

The original PAR-Q was developed by the British Columbia Ministry of Health. It has been revised by an Expert Advisory Committee assembled by the Canadian Society for Exercise Physiology and Fitness Canada (1994).

Disponible en français sous le titre «Questionnaire sur l'aptitude à l'activité physique - Q-AAP (revisé 1994)».

Supported by: Health Canada Santé Canada

Appendix E
Treatment Information Sheet

Ginseng and Exercise Study
Treatment Information Sheet

- Take one capsule three times daily for 35 days. Each capsule should be taken 40 minutes prior to a meal (i.e. breakfast, lunch, supper).

- Notify Sarah or Jennifer when you begin to run out of capsules. We have extra capsules for the end of the treatment periods.

- If you forget to take a capsule, take it as soon as you remember. It is very important that you consume three capsule each day.

- Avoid taking the capsules late at night.

- Store capsules in a dark, dry location at room temperature.

- Do not take the capsules with alcohol or drugs. This includes over the counter medications such as Tylenol or aspirin. If you must take a medication, do not take it with the treatment capsules. Record any medications you take and why. Notify Sarah or Jennifer as soon as possible.

- Avoid the use of other stimulants (i.e. caffeine), especially at the time of capsule consumption.

If you have any questions or concerns, please contact:
Sarah Robbins @ 492-4267 sarah@ualberta.ca or
Jennifer Humphreys @ 492-8739 jdh6@ualberta.ca

Appendix F
Adverse Effects Information

Ginseng and Exercise Study
Adverse Effects

It is possible that you will experience some side effects as a result of consuming ginseng. If you experience any of the following side effects, please record them on the attached form and contact the researchers. Side effects, if any, are likely to be transient.

Possible Side Effects Resulting from Ginseng Consumption:

- Nausea
- Vomiting
- Diarrhea
- Stomach pain or cramps
- Headache
- Anxiety
- Insomnia

To discuss any concerns that you may have, please contact:
Sarah Robbins @ 492-4267 sarah@ualberta.ca or
Jennifer Humphreys @ 492-8739 jdh6@ualberta.ca.

Ginseng and Exercise Study
Record of Adverse Experiences

Name:______________________________
Treatment Period:______________________
Group #:_____________________________

Please record any adverse experiences that you have throughout the treatment period. Please feel free to add any information that you feel is relevant. Indicate if you believe you experience is a result of an illness or of taking you supplement.

Description	**Date of Onset**	**Time**	**Severity**			**Duration**	**Notes**
Ex: Headache	August 26/99	Early morning	Mild	Moderate	Severe	Morning Only	

Appendix G
Sample Food Record

MENU ITEM			UNIT OF MEAS.	No. of Units	DESCRIPTION OF MENU ITEM		
Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating			Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"		Brand	Type of Flavour	Method of Cooking
MIDDAY MEAL	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Mark (X) One Category	Eaten at Your Home					
		Eaten Away From Your Home					
		Did Not Eat					

Day Two

MENU ITEM			UNIT OF MEAS.	No. of Units	DESCRIPTION OF MENU ITEM		
Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating			Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"		Brand	Type of Flavour	Method of Cooking
AFTERNOON SNACK	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Mark (X) One Category	Eaten at Your Home					
		Eaten Away From Your Home					
		Did Not Eat					

Day Two

Appendix H
Modified Baecke Questionnaire

Modified Baecke Questionnaire

Please circle or fill-in the appropriate response.

Question					
1. What is your main occupation?					
2. At work I sit…	Never	Seldom	Sometimes	Often	Always
3. At work I stand..	Never	Seldom	Sometimes	Often	Always
4. At work I walk…	Never	Seldom	Sometimes	Often	Always
5. At work I lift heavy loads…	Never	Seldom	Sometimes	Often	Always
6. At work I sweat…	Never	Seldom	Sometimes	Often	Always
7. After work I am tired…	Never	Seldom	Sometimes	Often	Always
8. In comparison with others of my own age, I think my work is physically…	much lighter	lighter	as heavy	heavier	much heavier
9. Do you play a sport?	Yes	No			
If yes:					
Which sport do you play most frequently?					
How many hours per week?	<1	1-2	2-3	3-4	>4
How many months per year?	<1	1-2	2-3	3-4	>4
If you play a second sport:					
Which sport is it?					
How many hours per week?	<1	1-2	2-3	3-4	>4
How many months per year?	<1	1-2	2-3	3-4	>4
10. In comparison with others my age, I think my physical activity during leisure time is…	much less	less	the same	more	much more
11. During leisure time, I sweat…	Never	Seldom	Sometimes	Often	Always
12. During leisure time, I play sports…	Never	Seldom	Sometimes	Often	Always
13. During leisure time, I watch TV	Never	Seldom	Sometimes	Often	Always
14. During leisure time, I walk…	Never	Seldom	Sometimes	Often	Always
15. During leisure time, I cycle…	Never	Seldom	Sometimes	Often	Always
16. During leisure time, I work in the garden…	Never	Seldom	Sometimes	Often	Always
17. During leisure time, I do do-it-yourself activities…	Never	Seldom	Sometimes	Often	Always
18. How many minutes per day do you walk and/or cycle to and from work, school, and shopping?	<5	5-15	15-30	30-45	>45
19. How many hours do you sleep (on average)?	<5	6	7	8	>9

Scoring for the Modified Baecke Questionnaire

1. Scoring for question #9:

A. Calculate Intensity

If type of Sport Equals	Then Intensity Equals
1	0.76
3	1.26
5	1.76

B. Calculate Time

If Number of Hours Per Week Equals	Then Time Equals
<1	0.5
1-2	1.5
2-3	2.5
3-4	3.5
>4	4.5

C. Calculate Proportion

If Number of Months Per Year Equals	Then Proportion Equals
<1	0.04
1-3	0.17
4-6	0.42
7-9	0.67
>9	0.92

D. Calculate Total Index for Question #9

Total = (Intensity1 x Time1 x Proportion1) + (Intensity2 x Time2 x Proportiond2)

E. Assign Likert Score

Total Score	Likert Score
0 (given to participants who do not play a sport)	1
0.01 - < 4	2
4 - < 8	3
8 - < 12	4
≥ 12	5

2. Calculation of the indices of physical activity:

Work Index = (1 + 2 + 3 + 4 + 5 + 6 + 7 + 8) / 8
Sports Index = (9 + 10 + 11 + 12) / 4
Leisure Time Index = (13 + 14 + 15 + 16 + 17 + 18 + 19) / 7
Total Activity Index = work index + sports index + leisure time index